(19) Eur päisch s P amt
European Patent Office
Office ur péen des br vets

(11) EP 0 811 687 A2

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
10.12.1997 Bulletin 1997/50

(21) Application number: **97303896.1**

(22) Date of filing: **06.06.1997**

(51) Int Cl.[6]: **C12N 15/55**, C12N 9/82, C12N 15/70, C12N 15/79, C12N 1/21, C12N 5/10, A61K 38/46 // (C12N1/21, C12R1:19)

(84) Designated Contracting States:
AT BE CH DE FR GB LI

(30) Priority: **07.06.1996 JP 168172/96**

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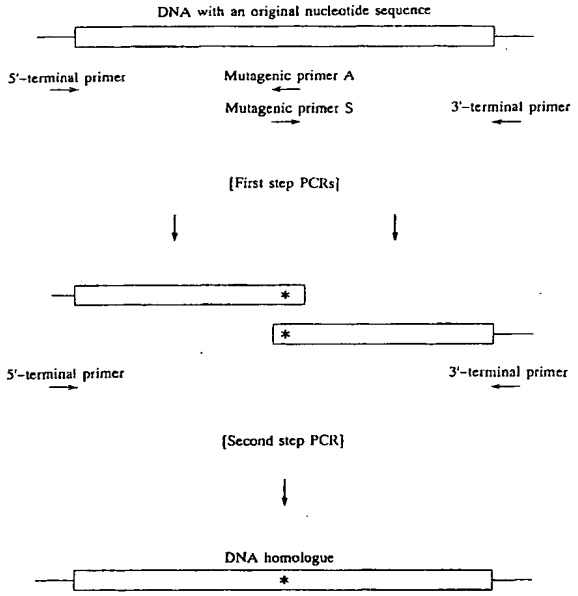
(54) Polypeptides having l-asparaginase activity

(57) Disclosed are polypeptides which originate from mammal, having L-asparaginase activity. The polypeptides are easily prepared by applying recombinant DNA techniques to DNAs encoding the polypeptides and they exert satisfactory effects in the treatment and/or the prevention for diseases caused by tumor cells dependent on L-asparagine, and cause no substantial serious side effects even when administered to humans in relatively-high dose.



Note: An asterisk indicates a site where a nucleotide is substituted, and a box indicates a polypeptide-encoding sequence.

FIG.1

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EP 0 811 687 A2

D scription

The present invention relates to L-asparagine amidohydrolytic enzymes, more particularly, to polypeptides which originate from mammal, having L-asparaginase activity.

L-Asparaginase (EC 3.5.1.1) is an enzyme which catalyzes the hydrolytic reaction of L-asparagine into L-aspartic acid and ammonia. The studies on the antitumor activity of L-asparaginase started from the following reports: J. G. Kidd et al. described the inhibitory action of guinea pig sera on cells of lymphomas in *"The Journal of Experimental Medicine"*, Vol.98, pp.565-582 (1953), and J. D. Broome et al. evidenced in *"Nature"*, Vol.191, pp.1,114-1,115 (1961), that the L-asparaginase activity of the guinea pig sera was responsible for the inhibitory action. It is now understood that the inhibitory action is caused by the lack of L-asparagine, an essential nutrient to proliferate and survive for some tumor cells which defect L-asparagine synthetase activity, such as acute lymphocytic leukemia, but not for normal cells. The hydrolysis of L-asparagine by L-asparaginase in patients with such tumor cells induces selective death of the tumor cells, resulting in the treatment of malignant tumors.

L-Asparaginase has been studied energetically for its actual use as an antitumor agent, and one derived from *Escherichia coli* is now in use as a therapeutic agent for leukemia and lymphoma. However, L-asparaginase from *Escherichia coli* is merely an external protein for human, and repetitive administration of conventional compositions with such L-asparaginase may cause serious side effects such as anaphylaxis shock, urticaria, edema, wheeze and dyspnea. These compositions are inevitably restricted with respect to administration dose and frequency. Therefore, some proposals to reduce or even diminish such side effects have been given.

As a first proposal, Japanese Patent Kokai No.119,082/79 discloses a chemically modified L-asparaginase from *Escherichia coli,* in which at least 65 % amino acids are blocked with 2-0- substituted polyethylene glycol-4,6-dichloro-S-triazine. As a second proposal, human L-asparaginases are disclosed in Japanese Patent Kokai Nos.320,684/92 and 19,018/80, where the L-asparaginases are respectively obtained from cultures of human cell lines and human urine. While the first proposal has an advantage of that the L-asparaginase from *Escherichia coli* is easily obtainable on an industrial scale, it has a disadvantage of that the modifying reaction is difficult to control and the side effects couldn't be eliminated completely. While the second proposal has an advantage of that unlike L-asparaginase from *Escherichia coli,* the L-asparaginases from human may not substantially induce antibodies even when administered to patients, it has a disadvantage of that it is not easy to obtain the L-asparaginases in a desired amount by the processes disclosed in Japanese Patent Kokai Nos.320,684/92 and 19,018/80.

Recently, recombinant DNA technology has advanced remarkably. If a DNA which encodes a desired polypeptide is once isolated, it is relatively easy to obtain a transformant which produces the polypeptide by constructing a recombinant DNA, comprising the DNA and a self-replicable vector, followed by introducing the recombinant DNA into a host, such as a microorganism, animal- or plant-cell. The polypeptide is obtainable in a desired amount from the culture of the transformant. However, no DNA which encodes mammalian L-asparaginase was isolated, and no mammalian L-asparaginase was produced by recombinant DNA techniques.

Therefore, it has been in great demand to isolate DNAs which encode active L-asparaginases originating from mammal and establish processes to prepare the L-asparaginases on a large-scale by applying the recombinant DNA techniques to the isolated DNAs.

In view of foregoing, the first object of the present invention is to provide a polypeptide which originates from mamma, having L-asparaginase activity.

The second object of the present invention is to provide a DNA which encodes the polypeptide.

The third object of the present invention is to provide a recombinant DNA which containing a DNA which encodes the polypeptide and a self-replicable vector.

The fourth object of the present invention is to provide a transformant obtainable by introducing a DNA which encodes the polypeptide into a host.

The fifth object of the present invention is to provide a process to prepare the polypeptide by using the transformant.

The sixth object of the present invention is to provide an agent for susceptive diseases, containing the polypeptide as an effective ingredient.

The first object of the present invention is attained by polypeptides which originate from mammal, having L-asparaginase activity.

The second object of the present invention is attained by DNAs which encode the polypeptides.

The third object of the present invention is attained by recombinant DNAs containing DNA which encode the polypeptides and a self-replicable vector.

The fourth object of the present invention is attained by transformants obtainable by introducing the DNAs into appropriate hosts.

The fifth object of the present invention is attained by a process to prepare the polypeptides which comprises culturing the transformants and collecting the produced polypeptides from the resultant cultures.

The sixth object of the present invention is attained by agents for susceptive diseases, containing the polypeptides

as effective ingredients.

FIG.1 is a scheme of the over lap extension method.

FIG.2 is a restriction map of the present recombinant DNA pKGPA/WT.

FIG.3 is a scheme of the preparation of the present recombinant DNA pBIgGPA/WT.

FIG.4 is a restriction map of the present recombinant DNA pBIgGPA/WT.

FIG.5 is a restriction map of the present recombinant DNA pKGPA/D364stp.

FIG.6 is a restriction map of the present recombinant DNA pKHA/MUT1.

FIG.7 is a restriction map of the present recombinant DNA pKHA/MUT2.

FIG.8 is a restriction map of the present recombinant DNA pKHA/MUT3.

FIG.9 is a restriction map of the present recombinant DNA pKHA/MUT3.

FIG.9 is a restriction map of the present recombinant DNA pKHA/MUT5.

FIG.10 is a restriction map of the present recombinant DNA pBIgGPA/D364stp.

FIG.11 is a restriction map of the present recombinant DNA pBIgHA/MUT1.

FIG.12 is a restriction map of the present recombinant DNA pBIgHA/MUT2.

FIG.13 is a restriction map of the present recombinant DNA pBIgHA/MUT3.

FIG.14 is a restriction map of the present recombinant DNA pBIgHA/MUT4.

Explanation of the symbols are as follows:

The symbols, *"Eco* RI", *"Hin* dIII", *"Not* I" and *"Xho* I", indicate cleavage sites by restriction enzymes, *Eco* RI, *Hin* dIII, *Not* I and *Xho* I, respectively.

The symbols, "D364stp", "HA/MUT1", "HA/MUT2", "HA/MUT3" and "HA/MUT5", indicate DNAs encoding the present polypeptides.

The symbol "Ptac" indicates a Tac promotor.

The symbol "rrnBTIT2" indicates a region for transcriptional termination, derived from a ribosomal RNA operon.

The symbol "AmpR" indicates an ampicillin resistant gene.

The symbol "pBR322ori" indicates a replication origin in *Escherichia coli.*

The symbol "Ig sec" indicates a DNA encoding a polypeptide with a signal sequence for secretion of immunoglobulin.

The symbol "Emsv" indicates an enhancer from long terminal repeats of Moloney Mouse Sarcoma Virus.

The symbol "Pmti" indicates a promotor for Mouse metallothionein I gene.

The symbol "Poly (A)" indicates a polyadenylation signal derived from SV40 virus.

The symbol "BPVI" indicates a genome of a bovine *papillomavirus.*

The present inventors isolated mammalian DNAs encoding L-asparaginases firstly in the world, from guinea pig and human, and succeeded in elucidating their nucleotide sequences. The nucleotide sequences of the DNAs from a guinea pig and human are in SEQ ID NOs:15 and 16, respectively. This information is disclosed in Japanese Patent Application No.42,564/95 (Japanese Patent Kokai No.214,885/96) by the same applicant of this application. The present invention has been made based on the above information, and provides the polypeptides which originate from mammal, having L-asparaginase activity.

The polypeptides of the present invention are not restricted to their sources or origins so far as they originate from mammal and have an L-asparaginase activity. The polypeptides are usually obtainable by the expression of genes originating from mammal, and usually contain amino acid sequences of SEQ ID NOs:1 to 3, wherein the symbol "Xaa" in SEQ ID NO:3 means "glutamine" or "arginine". For example, the polypeptides have any one of amino acid sequences of SEQ ID NOs:4 to 9. In view of the technical level in this field, one or more amino acid residues in SEQ ID NOs:4 to 9 can be substituted relatively easily by different ones without substantial defects of the activity. Despite derived from the same DNA, a variety of polypeptides with an L-asparaginase activity may be obtained as a result of modifications by endogenous enzymes of the hosts after the DNA expression or modifications during purification of the polypeptides, depending on the types of vectors and hosts used to obtain transformants or culturing conditions of the transformants, such as ingredients, compositions, temperatures or pHs. The wording "a variety of polypeptides" includes the polypeptides with deletions and/or additions of one or more amino acids at the N- and/or C-termini thereof, or with glycosylations. In view of these, the present polypeptides include not only the polypeptide with any amino acid sequence of SEQ ID NOs:4 to 9 but also their homologues so long as they have an L-asparaginase activity. The present polypeptides express the activity when exist in multiple forms, preferably, tetramers.

The polypeptides of the present invention can be usually prepared by the recombinant DNA techniques. In general, the polypeptides are obtainable by culturing transformants containing DNAs encoding the polypeptides and collecting the produced polypeptides from the resultant cultures. The transformants are obtainable by introducing such recombinant DNAs as contain any one of the nucleotide sequences of SEQ ID Nos:10 to 15 and a self-replicable vector into appropriate hosts. One or more nucleotides in SEQ ID NOs:10 to 15 can be substituted by different nucleotides without substantial changes of the encoding amino acid sequences with respect to degeneracy of genetic code. To facilitate the expression of the DNA in the hosts, one or more nucleotides in nucleotide sequences which encode the polypeptides

or their homologues can be appropriately substituted by different ones. Furthermore, nucleotide sequences which encode and/or don't encode one or more amino acids can be added to the 5'- and/or 3'-termini of the nucleotide sequences.

The DNAs encoding the polypeptides of this invention include those from natural sources and those by synthesized artificially so far as the polypeptides expressed by them have an L-asparaginase activity. The DNAs can be wild-type ones, containing the same nucleotide sequences as those from natural sources, and can be their homologues.

Examples of the wild-type DNAs include DNAs containing the nucleotide sequences of SEQ ID NOs:15. The wild-type DNA is obtainable from natural sources such as guinea pig livers, as disclosed in Japanese Patent Application No.42,564/95 (Japanese Patent Kokai No.214,885/96) by the same applicant of this invention: (a) constructing a cDNA library by applying usual methods to purified poly $(A)^+$ RNAs from a guinea pig or human liver as materials, (b) applying the plaque hybridization method to the cDNA library using oligonucleotides as probes synthesized chemically based on partial amino acid sequences of L-asparaginase purified from a guinea pig serum, (c) collecting phage clones containing the DNAs encoding the polypeptides of this invention, and (d) manipulating the collected phage clones in a conventional manner. The wild-type DNA can be synthesized chemically based on SEQ ID NO:15.

Examples of DNA homologues to the wild-type ones include DNAs containing any nucleotide sequence of SEQ ID NOs:10 to 14. DNA homologues containing the nucleotide sequence of SEQ ID NO:10 are obtainable by applying conventional methods in this field, such as PCR method and methods for site-directed mutagenesis, to the wild-type DNA of SEQ ID NO:15 concerning the desired sequence. DNA homologues containing any nucleotide sequence of SEQ ID NOs:11 to 14 are obtainable by the methods such as follows: Firstly, A wild-type DNA with the nucleotide sequence of SEQ ID NO:16 is obtained by the methods as disclosed in Japanese Patent Application No.42,564/95 (Japanese Patent Kokai No.214,885/96) by the same applicant of this invention, i.e., screening a human liver cDNA library. Subsequently, the wild-type DNA is subjected to conventional methods as mentioned above concerning desired sequences to obtain the DNA homologues. The DNA homologues can be synthesized chemically based on the nucleotide sequences of SEQ ID NOs:10 to 14.

The present DNAs can be generally introduced into hosts as in forms of recombinant DNAs. In general, each recombinant DNA comprises one of the present DNAs and a self-replicable vector. The recombinant DNAs can be easily prepared by general recombinant DNA techniques when the DNAs are available. Examples of such self-replicable vectors include pKK223-3, pGEX-2T, pRL-λ, pBTrp2 DNA, pUB110, YEpl3, Ti plasmid, Ri plasmid, pBI121, pCDM8, pBPV and BCMGSneo. Among these vectors, pKK223-3, pGEX-2T, pRL-λ, pBTrp2 DNA pUB110 are suitably used to express the present DNAs in prokaryotic cells such as *Escherichia coli* and *Bacillus* sp., while YEp13, Ti plasmid, Ri plasmid, pBI121, pCDM8, pBPV and BCMGSneo are suitably used to express the present DNAs in eukaryotic cells such as yeasts and animal- and plant-cells.

To insert the present DNAs into the vectors, conventional methods in this field can be arbitrarily used. Examples of such methods contain the steps of (a) cleaving self-replicable vectors with restriction enzymes, (b) introducing the same cleavage sites, by the same restriction enzymes as used to cleave the vectors, to the 5'- and 3'- termini of the present DNAs by applying polymerase chain reaction to form double-stranded DNAs, (c) cleaving the double-stranded DNAs by the restriction enzymes, and (d) ligating the cleaved vectors with cleaved DNAs by the action of DNA ligases. The recombinant DNAs thus obtained can be easily introduced into appropriate hosts, resulting in limitless replication of the DNAs by culturing the transformants.

The recombinant DNAs according to the present invention can be introduced into appropriate hosts such as *Escherichia coli, Bacillus* sp., actinomycetes, yeasts and plant- and animal-cells. To introduce the DNAs into *Escherichia coli,* it can be cultured in the presence of the recombinant DNAs and calcium ion. To introduce them into *Bacillus* sp., competent cell methods or protoplast methods can be used. To introduce them into animal-cells, DEAE-dextran methods or electroporation methods can be used. Desired transformants can be cloned by applying hybridization methods or by selecting L-asparaginase producing cells from the cultures.

The transformants thus obtained produce the present polypeptides intracellularly or extracellularly when cultured in nutrient culture media. Examples of such media are usually liquid nutrient culture media which generally contain carbon sources, nitrogen sources and minerals, and further contain micronutrients such as amino acids and/or vitamins on demand. The carbon sources usable in the present invention include saccharides such as starch, starch hydrolysates, glucose, fructose and sucrose. The nitrogen sources usable in the present invention include organic and inorganic compounds containing nitrogen, such as ammonia and their salts, urea, nitrates, peptone, yeast extract, defatted soy bean, corn steep liquor and beef extract. Cultures containing the present polypeptides can be obtained by inoculating the transformants into the above media, culturing them at temperatures of 25-65°C at pHs of 5-8 for about 1-10 days under aerobic conditions by aeration-agitation method, etc.

The cultures can be used intact as agents for susceptive diseases. However, the cultures are usually treated with ultrasonication or cell wall lytic enzymes to disrupt cells, and the present polypeptides are separated by using techniques such as filtration and centrifugation from the cell-disruptants and purified. Alternatively, the polypeptides can be purified from the culture supernatants obtained by removing cells from the cultures by filtration or centrifugation,

etc. The present polypeptides can be purified by applying techniques generally used in this field for protein purifications, such as salting out, dialysis, filtration, concentration, gel filtration chromatography, ion-exchange chromatography, affinity chromatography, hydrophobic chromatography, isoelectric focusing and gel electrophoresis, and if necessary, two or more of them can be applied combination to the supernatants which are separated from insoluble substances of cell-disruptants, or to the culture supernatants. The resultant purified solutions polypeptides can be concentrated and/or lyophilized into liquids or solids depending on their final uses.

The following experiments explain the present invention in more detail, and the techniques used therein are conventional ones in this field: For example, the techniques are disclosed by J. Sambrook et al. in *"Molecular Cloning, A Laboratory Manual"*, 2nd edition (1989), published by Cold Spring Harbor Laboratory Press, New York, U.S.A., and by Masami MATSUMURA in *"Laboratory Manual for Genetic Engineering"* (1988), published by Maruzen Co., Ltd., Tokyo, Japan.

Experiment 1

Expression of wild-type DNA

Experiment 1-1

Expression of guinea pig wild-type DNA

Experiment 1-1(a)

Preparation of guinea pig wild-type DNA

A guinea pig wild-type DNA encoding L-asparaginase was prepared by the method disclosed in Japanese Patent Kokai No.214,885/96 by the same applicant of this invention. The DNA had the nucleotide sequence of SEQ ID NO: 15. A DNA having a polypeptide-encoding region in SEQ ID NO:15, i.e., a sequence of containing the nucleotides 20-1,714 in SEQ ID NO:15, is called "GPA/WT DNA" hereinafter, and the expression product thereof with the amino acid sequence of SEQ ID NO:15 is called "guinea pig wild-type L-asparaginase". SEQ ID NO:17 shows in parallel the nucleotide sequence of GPA/WT DNA and the amino acid sequence encoded thereby.

Experiment 1-1(b)

Preparation of recombinant DNA

Ten μl of 10 x PCR buffer, one μl of 25 mM dNTP mix, one ng of the human wild-type DNA, obtained in Experiment 1-1(a), as a template were placed in 0.5 ml reaction tube. The mixture was mixed with, as a sense- and anti-sense-primers, an adequate amount of an oligonucleotide chemically synthesized based on the amino acid sequences near the N- and C- termini of SEQ ID NO:15, volumed up with sterilized distilled water to give a total volume of 99.5 μl, and mixed with 0.5 μl of 2.5 units/μl of AmpliTaq DNA polymerase. The nucleotide sequence of the sense primer was 5'-AATCTCGAGCCACCATGGCGCGCGCATCA-3', a nucleotide sequence obtained by adding a common nucleotide sequence in animal cells, as shown by M. Kozak in *"Nucleic Acid Research"*, Vol.15, pp.8,125-8,148 (1987), to the upstream of a region which encodes the N-terminal amino acid sequence of SEQ ID NO:15 and then adding to the further upstream a cleavage site by a restriction enzyme, *Xho* I. The nucleotide sequence of the anti-sense primer was 5'-CTGCGGCCGCTTATCAGATGGCAGGCGGCAC-3' as a complement to a nucleotide sequence obtained by adding two termination codons to the downstream of a region which encodes the C-terminus of the amino acid sequence of SEQ ID NO:15 and adding a cleavage site by a restriction enzyme, *Not* I, to the further downstream. The resulting mixture was successively incubated at 940C for one min, at 55°C for one min, and at 72°C for 3 min, and the series of incubation was repeated 40-times for PCR to amplify DNA. Thus, a DNA containing GPA/WT DNA was obtained and then cleaved by restriction enzymes of *Xho I* and *Not* I to obtain an about 1.7 kbp DNA fragment. Twenty-five ng of the DNA fragment was weighed and mixed with 10 ng of a plasmid vector, "pCDM8", commercialized by Invitrogen Corporation, San Diego, U.S.A., which had been cleaved by restriction enzymes of *Xho* I and *Not* I. To the DNA mixture thus obtained was added an equal volume of the solution I in "LIGATION KIT VERSION 2" commercialized by Takara Shuzo, Tokyo, Japan, and incubated at 160C for 2 hours to obtain a replicable recombinant DNA, "pCGPA/WT".

The recombinant DNA pCGPA/WT was introduced into an *Escherichia coli* MC1061/P3 strain, commercialized by Invitrogen Corporation, San Diego, U.S.A., by competent cell method. The transformant thus obtained was inoculated into L broth medium (pH 7.2) containing 20μg/ml ampicillin and 10 μg/ml tetracycline followed by cultivation at 37°C for 18 hours under shaking conditions. The transformants were collected from the culture by centrifugation and sub-

jected to conventional alkali-SDS method to extract the recombinant DNA pCGPA/WT. The analysis of the pCGPA/WT by an automatic sequencer equipped with a fluorophotometer confirmed that it contained GPA/WT DNA, which termination codons were ligated to the 3'-terminus and was ligated to the downstream of a CMV promotor from the 5'- to 3'-termini.

The system using COS-1 (ATCC CRL-1650) as a host, which is a cell line derived from a monkey kidney, was used to express the DNA in the following Experiments 1 and 2. Since the system is for a transient expression, it has a disadvantage that DNAs introduced into transformants could not be stable over several days, and the transformants do not produce the desired polypeptides repeatedly. However, it is known that the number of copies of the desired DNA per cell temporally increases to 10^5 when plasmid vectors having a replication origin derived from SV40 virus, such as the above mentioned pCDM8, are introduced into the COS-1 cells. With this point of view, the system has a merit that it quite easily analyzes the desired DNA-expression product.

Experiment 1-1(c)

Recombinant DNA expression in COS-1 cell

In accordance with the DEAE-dextran method reported by Frederick M. Ausubel et al. in *"Current Protocols in Molecular Biology"* (1987), chapters 9.2.1-9.2.3 and 9.2.5-9.2.6, published by John Wiley and Sons Inc., New York, U. S.A., the recombinant DNA pCGPA/WT in Experiment 1-1(b) was introduced into COS-1 cells for its expression. To each well of "3046", a plastic multiwell plate, with 6 wells of 3.5 cm diameter, commercialized by Becton Dickinson Labware, New Jersey, U.S.A., was added 2.5 ml of DME medium, containing 10 v/v % bovine fetal serum and 1.8 x 10^5 COS-1 cells. The cells were cultured at 370C in a 5 v/v % CO_2 incubator overnight. After removing the culture supernatant by an aspirator and washing the remaining cells with DME medium containing 50 mM Tris-HCl buffer (pH 7.4), each well was charged with 2.5 ml of DME medium containing 2.8 μg/ml PCGPA/WT, 50 Mm Tris-HCl (pH 7.4), 0.4 mg/ml DEAE-dextran and 0.1 mM chloroquine, and incubated at 37°C for 4 hours in a 5 v/v % CO_2 incubator. Thereafter, the culture supernatant was removed, and the remaining cells in each well were received with 2.5 ml of 10 mM phosphate buffered saline (hereinafter abbreviated as "PBS") containing 10 v/v % DMSO before incubating at ambient temperature for 2 minutes. After removing the supernatant and washing the remaining cells with DME medium containing 50 mM Tris-HCl (pH 7.4), each well was charged with 2.5 ml of "COS MEDIUM", commercialized by COSMO BIO CO. LTD., Tokyo, Japan, followed by cultivation at 37°C for 3 days in a 5 v/v % CO_2 incubator to express the desired DNA. As a control, the same experiment was carried out using a plasmid vector, pCDM8.

After 3 days' cultivation, the multiwell plates with the cultures were subjected thrice to a treatment of freezing at -800C and thawing at ambient temperature to disrupt the cells. The whole cultures were transferred to centrifugal tubes and centrifuged to remove insoluble components after precipitated, followed by obtaining total soluble fractions, concentrating the fractions using membranes, and adjusting the volume of the total soluble fraction per well to give 0.5 ml for the following analyses.

Experiment 1-1(d)

Assay for L-asparaginase activity

L-Asparaginase activity was expressed by the unit assayed as follows: Samples were placed in 1.5 ml-reaction tubes in 50 μl each and admixed with 200 μl of 50 mM phosphate buffer (pH 7.0) containing 1.4 mg/ml L-asparagine. After standing at 37°C for 0, 1, 2, 4, 6 and 16 hours, L-aspartic acid in the reaction mixtures was quantified by an amino acid analyzer. In parallel, 1.0, 0.5 and 0.25 unit/ml dilutions of an L-asparaginase from *Escherichia coli* were provided and quantified for L-aspartic acid after incubating at 37°C for 0 and one hour, and based on the increased amount of L-aspartic acid, a calibration curve was drawn. By plotting on the calibration curve the increased amounts of L-aspartic acid of the samples, the samples' L-asparaginase activities were estimated. The activity of samples with a lower activity was estimated based on that assayed after 2 hours or more incubation. One unit activity of L-asparaginase was defined as the amount that releases one μmol of ammonia from L-asparagine per minute under the above conditions.

The total soluble fractions obtained in Experiment 1-1(c) were treated similarly as above, and expressed their activities as total L-asparaginase activities that were detected in the soluble fractions from 1.8 x 10^5 COS-1 cells. As a result, the activity of the total soluble fraction in Experiment 1-1(c) was 0.083 unit, and the control gave no activity.

Experiment 1-1(e)

Western blotting

An anti-L-asparaginase antibody was prepared as follows: An oligopeptide of a sequence Gly-Ser-Gly-Asn-Gly-Pro-Thr-Lys-Pro-Asp-Leu-Leu-Gln-Glu-Leu-Arg-Cys was synthesized chemically in a usual manner. Keyhole Limped Hemocyanin was linked to the C-terminus of the oligopeptide. The resultant was purified and used to immunize rabbits in a usual manner. The rabbits were immunized 6 times 2 weeks about, then the whole blood was collected and subjected to salting out with 50 w/v % ammonium sulfate to obtain an anti-L-asparaginase anti-serum.

In accordance with the method reported by U. K. Laemli et al. in *"Nature"*, Vol.227, pp.680-685 (1970), 0.2 ml of the total soluble fraction in Experiment 1-1(c) was subjected to 12.5 w/v % SDS-polyacrylamide gel electrophoresis (hereinafter abbreviated as "SDS-PAGE"). The polypeptides migrated were transferred to a nitrocellulose membrane and subjected to Western blotting using the above anti-L-asparaginase anti-serum, in accordance with the method reported by H. Towbin in *"Proceedings of the National Academy of Sciences of the U.S.A."*, Vol.76, pp.4,350-4,354 (1979). For color development, alkaline phosphatase system was used. Comparing with the control and molecular weight markers, both the identification of bands specifically stained in the sample and the measurement of the molecular weight of each subunit of the L-asparaginase were carried out. The molecular weight markers used were bovine serum albumin (67 kDa), ovalbumin (45 Kda), soy bean trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa), and stained with amide black. The total soluble fraction in Experiment 1-1(c) gave no clear band.

Experiment 1-1(f)

Measurement of molecular weight on gel filtration

Two ml of the total soluble fraction in Experiment 1-1(c) was subjected to gel filtration column chromatography using "HILOAD SUPERDEX 200 COLUMN", with an inner diameter of 16 mm and a length of 60 cm, commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, equilibrated with PBS. Based on the L-asparaginase activity of the eluted fractions, the molecular weight of the guinea pig wild-type L-asparaginase in a native form was examined. The molecular weight markers used were thyroglobulin (699 kDa), ferritin (440 kDa), catalase (232 kDa), aldolase (158 kDa), bovine serum albumin (67 kDa) and ovalbumin (43 kDa). The peak of L-asparaginase activity in the eluted fractions was observed in a position corresponding to a molecular weight of about 300 kDa.

Since no clear band was detected by Western blotting, the molecular weight of the wild-type L-asparaginase in a dissociated form could not be detected, while the molecular weight in a native form was estimated to be about 300 kDa based on the result of gel filtration. The molecular weights of L-asparaginase in a native and dissociated form, purified from guinea pig L-asparaginase in serum, were respectively estimated to be about 190 kDa on gel filtration and about 43 kDa on DS-PAGE. As disclosed in Japanese Patent Kokai No.214,885/96 by the same applicant of the present invention, 3 partial amino acid sequences of a guinea pig L-asparaginase in serum were observed in a region of amino acids 10-236 in the sequence of guinea pig wild-type L-asparaginase. While, two consensus amino acid sequences essential for the expression of L-asparaginase activity, i.e., SEQ ID NOs:1 and 2, as proposed by E. Harms in *"FEBS letters"*, Vol.285, pp.55-58 (1991) based on the results of experiments on L-asparaginase derived from *Escherichia coli,* correspond to the sequences of amino acids 16-19 and 114-118 in the amino acid sequence of the guinea pig wild-type L-asparaginase. In view of these and the results in Experiment 1-1, the present inventors estimated that the guinea pig wild-type L-asparaginase may require a region of amino acids about 1-400 in the amino acid sequence to express the activity. In Experiment 2-1, to examine the L-asparaginase activities of C-terminal defective mutants as homologues of the guinea pig wild-type L-asparaginase, the expression products of DNA homologues from a guinea pig were tested for properties and features.

Experiment 1-2

Expression of human wild-type DNA

A human wild-type DNA encoding L-asparaginase was prepared according to the method in Japanese Patent Kokai No.214,855/96 by the same applicant of the present invention. The DNA had the nucleotide sequence of SEQ ID NO:16. Hereinafter, a DNA having a polypeptide-encoding region in SEQ ID NO:16, i.e., a sequence of nucleotides 93-1,811 in SEQ ID NO:16, was named "HA/WT DNA", and a polypeptide, as the expression product of HA/WT DNA, having the amino acid sequence of SEQ ID NO:16, may be called "human wild-type L-asparaginase". SEQ ID NO:18 shows the nucleotide sequence of GPA/WT DNA and the amino acid sequence encoded thereby.

Except for the template and the sense- and anti-sense-primers, PCR was performed under the same conditions

as used in Experiment 1-1(b). As a template, the human wild-type DNA in Experiment 1-2 was used. As a sense- and anti-sense-primers, oligonucleotides with sequences of 5'-AATCTCGAGCCACCATGGCGCGCGCG GTG-3' and 5'-CTGCGGCCGCTTATCAGACACCAGGCAGCAC-3' were respectively used. The DNA thus amplified was continuously treated with the same method as used in Experiment 1-1(b) to prepare a recombinant DNA, "pCHA/WT". After sequencing, the pCHA/WT was introduced into COS-1 cells and expressed followed by analyzing the expression product similarly as in Experiment 1-1.

In contrast to the guinea pig wild-type L-asparaginase, the experiment system could not detect the human wild-type L-asparaginase activity. It was presumably due to that the human wild-type L-asparaginase had a lower specific activity than that of the guinea pig wild-type one, and this forced to examine the properties of expression products by DNA homologues from human in Experiment 2-2.

Experiment 2

Expression of DNA homologue

Experiment 2-1

Expression of DNA homologue originating from guinea pig

A termination codon was replaced for the nucleotide sequence in a specific position of the guinea pig wild-type DNA to obtain a DNA homologue: A DNA was obtained by PCR method by replacing a termination codon for a codon of the nucleotides 1,090-1,092 or 1,012-1,014 in SEQ ID NO:17. Except for the nucleotide sequence of anti-sense primer, PCR was performed under the same conditions as used in Experiment 1-1(b). As an anti-sense primer, an oligonucleotide with a sequence of 5'-CTGCGGCCGCTTATCATGCCGTGGGCAGTGT-3'or 5'-CTGCGGCCGCTTATCAGCCCAACACGTAGGA-3' was used to prepare the two-types of DNAs. The amplified DNAs were treated similarly as in Experiment 1-1(b) to obtain recombinant DNAs, "pCGPA/D364stp" and "pCGPA/L338stp". By sequencing similarly, it was confirmed that pCGPA/D364stp and pCGPA/L338stp had DNAs, encoding the sequences of amino acids 1-363 and 1-337 in the guinea pig wild-type L-asparaginase, respectively, and had a termination codon at their 3'-termini free of intervening sequences. Hereinafter, the polypeptide-encoding regions of the DNAs are respectively named "GPA/D364stp DNA" and "GPA/L338stp DNA". GPA/D364stp DNA and GPA/L338stp DNA were ligated in the downstream of a CMV promoter in the direction from the 5'- to 3'-termini. The DNAs expression products may be named "guinea pig L-asparaginase homologues".

The above recombinant DNAs were introduced into COS-1 cells and examined similarly as in Experiment 1-1. As controls, pCGPA/WT and pCDM8 in Experiment 1-1(b) were similarly treated and examined. Table 1 shows the results.

Table 1

Recombinant DNA	L-asparaginase activity (unit)	Molecular weight (kDa) *1	Molecular weight (kDa) *2
pCGPA/WT	0.083	-	about 300
pCGPA/D364stp	0.228	about 40	about 140
pCGPA/L338stp	N.D. *3	about 40	-
pCDM8	N.D. *3	-	-

Note: The symbols "*1", "*2" and "*3" mean that the value was determined by Western blotting, the value was determined by gel filtration, and the activity was not detected, respectively.

As shown in Table 1, the activities of the expression products of GPA/WT DNA and GPA/D364stp DNA were detected, but not for GPA/L338stp DNA. These results suggest that a region of amino acids 1-363 in the guinea pig wild-type L-asparaginase may be enough for sufficiently expressing the L-asparaginase activity. This amino acid sequence, amino acids 1-363 in the guinia pig wild-type, is SEQ ID NO:4, and a nucleotide sequence which encodes the

amino acid sequence is SEQ ID NO:10. The amino acid sequence of the guinea pig wild-type L-asparaginase is SEQ ID NO:5.

Experiment 2-2

Expression of DNA homologue originating from human

DNA homologues were prepared by replacing specific codons in the human wild-type DNA with termination codons or codons for different amino acids: The DNA homologues were prepared by replacing termination codons for the nucleotides 1096-1098 in SEQ ID NO:18 by applying PCR method. Except for the template and the sense- and anti-sense-primers, PCR was performed under the same conditions as used in Experiment 1-1(b). As a template, the human wild-type DNA in Experiment 1-2 was used. As a sense- and anti-sense-primers, the oligonucleotides with sequences of 5'-AATCTCGAGCCACCATGGCGCGCGCGGTG-3' and 5'-CTGCGGCCGCTCATTACACCGAGGGTGGCGT-3' were respectively used. The amplified DNA was treated similarly as in Experiment 1-1 to obtain a recombinant DNA, "pCHA/E366stp", and sequenced. It was confirmed that pCHA/E366stp contained a DNA encoding amino acids 1-365 in SEQ ID NO:16 and a termination codon at the 3'-terminus free of intervening sequences. The polypeptide-encoding region was named "HA/E366stp DNA", hereinafter. HA/E366stp DNA was ligated to the downstream of a CMV promotor in the direction from the 5'- to 3'-termini.

To change specific codons in DNAs into ones for different amino acids, the over lap extension method reported by Robert M. Horton et al. in *"Methods in Enzymology"*, Vol.217, pp.270-279 (1993), published by Academic Press, Inc., San Diego, U.S.A., was used. The method is summarized in FIG.1 and explained as follows: First, mutagenic primers A and B, where the nucleotides to be mutagenized were substituted by desired different ones complementary to one another, were prepared. The mutagenic primer A was a sense strand, and the mutagenic primer B was an anti-sense strand. A set of 5'- and 3'-terminal primers, which amplify the whole region of the desired DNA, were prepared, and they were respectively a sense- and anti-sense-strands. Second, conventional PCR was performed using the 5'-terminal primer, the mutagenic primer A, and as a template, a DNA with the original nucleotide sequence. In parallel, another PCR as was performed using the same DNA as a template, the 3'-terminal primer, and the mutagenic primer B. These two PCRs were named "first step PCRs". Third, two DNAs amplified in the first step PCRs were mixed with the 5'- and 3'-terminal primers as used in the first step PCRs followed by performing PCR as a second step PCR. The two DNA fragments amplified in the first step PCRs were used as primers and templates to generate mutagenized DNAs, while the 5'- and 3'-terminal primers were used as primers to amplify the mutagenized DNAs. By this method, DNAs into which were introduced 7 types nucleotide substituents, i.e., 7 DNA homologues were prepared. The 7 types nucleotide substituents and consequent changes of the encoded amino acid sequences are summarized in Table 2. The template DNA and mutagenic primers A and B used to prepare the 7 DNA homologues were summarized in Table 3. The 5'- and 3'-terminal primers were respectively equal to the sense- and anti-sense-primers as used to prepare pCHA/E366stp in Experiment 2-2.

Table 2

DNA homologue	Recombinant DNA	Nucleotide substitution (upper line) and consequential change of amino acid (lower line) *
HA/MUT1 DNA	pCHA/MUT1	C894G, A902G, G952A, G953A and G1096T H298Q, Q301R, G318N and E366stp
HA/MUT2 DNA	pCHA/MUT2	C894G, A902G and G1096T H298Q, Q301R and E366stp
HA/MUT3 DNA	pCHA/MUT3	C894G, G952A, G953A and G1096T H298Q, G318N and E366stp
HA/MUT4 DNA	pCHA/MUT4	A902G, G952A, G953A and G1096T Q301R, G318N and E366stp
HA/MUT5 DNA	pCHA/MUT5	C894G and G1096T H298Q and E366stp
HA/MUT6 DNA	pCHA/MUT6	A902G and G1096T Q301R and E366stp
HA/MUT7 DNA	pCHA/MUT7	G952A, G953A and G1096T G318N and E366stp

*) Numbers in the upper lines in each column mean a nucleotide number in SEQ ID NO:18. Numbers in the lower lines in each column means an amino acid residue number in SEQ ID NO:18. Alphabets on the left and right of the numbers in the upper lines show nucleotides before and after the nucleotide substitution, respectively. Alphabets on the left and right of the numbers in the lower lines show amino acids before and after the nucleotide substitution, respectively. The symbol "stp" means that a termination condon was substituted for a codon in the wild-type DNA. Names for the 7 DNA homologues and the recombinant DNAs containing the DNA homologues are shown in parallel.

Table 3

DNA homologue	Template DNA	Nucleotide sequences of mutagenic primers A (upper line) and B (lower line) *
HA/MUT1 DNA	pCHA/MUT7	the same as used for HA/MUT2 DNA preparation the same as used for HA/MUT2 DNA preparation
HA/MUT2 DNA	pCHA/E366stp	5'-CCCCcGGAGGCAcTGGGT-3' 5'-ACCCAgTGCCTCCgGGGG-3'
HA/MUT3 DNA	pCHA/MUT7	the same as used for HA/MUT5 DNA preparation the same as used for HA/MUT5 DNA preparation
HA/MUT4 DNA	pCHA/MUT7	the same as used for HA/MUT6 DNA preparation the same as used for HA/MUT6 DNA preparation
HA/MUT5 DNA	pCHA/E366stp	5'-CCCCTGGAGGCAcTGGGT-3' 5'-ACCCAgTGCCTCCAGGGG-3'
HA/MUT6 DNA	pCHA/E366stp	5'-CCCCcGGAGGCAGTGGGT-3' 5'-ACCCACTGCCTCCgGGGG-3'
HA/MUT7 DNA	pCHA/E366stp	5'-GACGttGGCTCCCGCCAT-3' 5'-ATGGCGGGAGCCaaCGTC-3'

Note: Small letters mean nucleotides which were substituted for those in human wild-type DNA.

The obtained DNA homologues from human were treated similarly as in Experiment 1-1 to obtain recombinant DNAs "pCHA/MUT1", "pCHA/MUT2", "pCHA/MUT3", "pCHA/MUT4", "pCHA/MUT5", "pCHA/MUT6" and "pCHA/MUT7". The expression products of the DNA homologues, obtained in Experiment 2-2, may be named "human L-

asparaginase homologues", hereinafter. After sequencing, these DNA homologues were introduced into COS-1 cells, followed by expression and assay. As controls, pCHA/WT obtained in Experiment 1-2 and pCDM8 were treated and examined. Signal intensities of bands, detected by Western blotting, were evaluated by densitometry to compare quantitatively the expressed products. The results were in Table 4.

Table 4

Recombinant DNA	L-asparaginase activity (unit) *1	Molecular weight (kDa) *2	Quantity *3	Molecular weight (kDa) *4
pCHA/WT	N.D.	-	-	-
pCHA/E366stp	N.D.	about 40	2.3	-
pCHA/MUT1	0.021	about 40	0.4	about 140
pCHA/MUT2	0.031	about 40	0.9	about 140
pCHA/MUT3	0.009	about 40	0.1	about 140
pCHA/MUT4	N.D.	about 40	0.2	-
pCHA/MUT5	0.006	about 40	1.2	about 140
pCHA/MUT6	N.D.	about 40	1.9	-
pCHA/MUT7	N.D.	about 40	0.2	-
pCDM8	N.D.	-	-	-

Note: The symbols "*1", "*2", "*3" and "*4" mean the activity was not detected, the value was determined by Western blotting, the value indicates the signal intensity of the band detected on Western blotting and quantified by densitometry, and the value was determined by gel filtration, respectively.

The results in Table 4 indicate that human L-asparaginases both in the wild-type and in the C-terminal defected mutant, i.e., the expression product of HA/E366stp DNA, as the one of the homologues, had a lower specific activity than that from guinea pigs. In addition, these results indicate that the specific activity of L-asparaginases among those of point mutants, which some of the amino acids inherent to the human wild-type L-asparaginase were substituted by different ones, increased to a detectable level. The human DNA homologues such as HA/MUT1, HA/MUT2, HA/MUT3 and HA/MUT5, which the expression products gave a detectable level of activity, have SEQ ID NOs:11 to 14, respectively, and encoding SEQ ID NOs:6 to 9, respectively.

Based on the results in the above experiments, the present inventors found that polypeptides from mammal may require the amino acid sequence of SEQ ID NO:3 (where the symbol "Xaa" meant "glutamine" or "arginine") to express a detectable level of L-asparaginase activity in the expression and assay systems in Experiments 1 and 2, in addition to conventionally known as such amino acid sequences of SEQ ID NOs:1 and 2. The animo acid sequence of the guinea pig wild-type L-asparaginase contains the SEQ ID NO:3 in the region the amino acids 298-302. Examples of such polypeptides, having all the amino acid sequences of SEQ ID NOs:1 to 3, include those having SEQ ID NOs:4 and 5 from guinea pigs and those having SEQ ID NOs:6 to 9 from human.

Based on the above findings, the present inventors invented the polypeptides having L-asparaginase activity. The following examples explain the present invention, and the techniques used therein are conventional ones used in the art, and of course, they are not restrictive to the present invention: Example A-1 Polypeptides having L-asparaginase activity Example A-1(a)

Preparation of transformant

Ten µl of 10 x PCR buffer, one µl of 25 mM dNTP mix, one ng of the recombinant DNA pCGPA/WT DNA obtained in Experiment 1-1 as a template, and an adequate amount of oligonucleotides as a sense- and anti-sense-primers synthesized chemically based on the 5'- and 3'-terminal sequences of GPA/WT DNA were placed in 0.5 ml reaction tube. The mixture was mixed with sterilized distilled water to give a total volume of 99.5 µl, and 0.5 µl of 2.5 units/µl AmpliTaq DNA polymerase were further added. The sequence of the sense primer was 5'-GCGAATTCATGGCGCGCG-CATCA-3' which was a nucleotide sequence obtained by adding a cleavage site by a restriction enzyme, *Eco* RI, to the upstream of the 5'-terminus of GPA/WT DNA. The sequence of the anti-sense primer was 5'-GCAAGCTTTCA-GATGGCAGGCGGCAC-3', which was complementary to a nucleotide sequence prepared by adding a termination codon to the 3'-terminus of GPA/WT DNA and then adding a cleavage site by a restriction enzyme, *Hin* dIII, to the downstream. The above mixture was subjected to 40 cycles of successive incubations at 940C for one min, at 55°C for one min, and 72°C for 3 min to perform PCR. By cleaving the amplified DNA by restriction enzymes *Eco* RI and *Hin* dIII, a *Eco* RI-*Hin*-dIII fragment with a length of about 1.7 kbp was obtained. Twenty-five ng of the DNA was mixed with 10 ng of plasmid vector "pKK223-3", commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, which had been cleaved by restriction enzymes *Eco* RI and *Hin* dIII, and then mixed with the solution I in "LIGATION KIT VERSION 2" commercialized by Takara Shuzo Inc., Tokyo, Japan, in an equal volume of the DNA mixture, followed by incubation at 160C for 2 hours to obtain a replicable recombinant DNA, "pKGPA/WT".

The recombinant DNA pKGPA/WT was introduced into an *Escherichia coli* strain "JM105" by the competent cell method. The resulting transformant "J-GPA/WT" was inoculated to L broth medium (pH 7.2) containing 50 µg/ml ampicillin and cultured at 37°C for 18 hours under shaking conditions. The transformants collected by centrifugation from the culture were subjected to a conventional alkali-SDS method to extract the recombinant DNA pKGPA/WT. As shown in FIG.2, analysis using an automatic sequencer equipped with a fluorophotometer revealed that GPA/WT DNA of SEQ ID NO:17 ligated to the downstream of a Tac promotor in the direction from the 5'- to 3'-termini. In addition, it was confirmed that a termination codon was ligated to the downstream of GPA/WT DNA without intervening sequences.

Example A-1(b)

Production of polypeptide

The transformant J-GPA/WT was inoculated into L broth medium (pH 7.2), containing 50 µg/ml ampicillin, and cultured at 37°C for 18 hours under shaking conditions to obtain a seed culture. Eighteen L of a fresh preparation of the same medium was placed in a 30-L jar fermenter, inoculated with one v/v % of the seed culture, and cultured at 37°C under aeration-agitation conditions. A portion of the culture was placed in a cuvette with 1-cm in thickness, incubated until the absorbance at a wavelength of 650 nm reached to about 1.5, admixed with IPTG to give a final concentration of 0.1 mM, and incubated for 5 hours. The cells centrifugally collected from the culture were suspended in a mixture solution (pH 7.2) containing 139 mM NaCl, 7 mM Na_2HPO_4 and 3 mM NaH_2PO_4, and supersonicated to disrupt the cells, followed by centrifuging the resultant to obtain a supernatant.

Ammonium sulfate was added to the supernatant under ice-chilling conditions to give a concentration of 50 w/v %

and then dissolved to homogeneity. After standing for several minutes, the precipitates were collected by centrifugation, dissolved in 20 mM Tris-HCl buffer (pH 8.0), and dialyzed against a fresh preparation of the same buffer followed by applying the dialyzed solution to "Q SEPHAROSE FF COLUMN", commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, equilibrated with the same buffer. After washing sufficiently with the same buffer, the column was fed with a linear gradient buffer of NaCl increasing from 0 M to 0.5 M in 20 mM Tris-HCl buffer (pH 8.0). The fractions eluted at about 0.1-0.3 M NaCl were collected, and the solvent was replaced with 10 mM sodium-phosphate buffer (pH 7.5) while concentrating with membranes. The concentrated solution was then applied to "L-ASPARAGINE AGAROSE", commercialized by Sigma Chemical Co., St. Louis, U.S.A., equilibrated with the same buffer. After washing with the same buffer, 10 mM sodium phosphate buffer (pH 7.5) containing 0.5 M NaCl was fed to the column for elution. The eluted fractions were pooled and concentrated by using a membrane. The concentrate was applied to "HILOAD SUPERDEX 200 COLUMN", commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, equilibrated with Tris-HCl buffer (pH 8.0) containing 10 v/v % glycerol, and eluted from the column. The eluted fractions, containing substances with a molecular weight of about 300 kDa, were collected to obtain a purified polypeptide with a purity of 90% or more in a yield of about 0.1 mg/ml culture.

Example A-1(c)

Physicochemical property of polypeptide

The purified polypeptide in the above was analyzed to determine the physicochemical properties: The molecular weight of the purified polypeptide in a native form was determined by gel filtration similarly as in Experiment 1-1(e). The peak for L-asparaginase activity of the eluted fractions was found at a position corresponding to a molecular weight of about 300 kDa. The molecular weight of the purified polypeptide in a dissociated form was determined by SDS-PAGE as used in Experiment 1-1(e). The main band was observed at a position corresponding to a molecular weight of about 50±10 kDa. The results indicate that the purified polypeptide exists in a multimer as a native form. Considering errors in measurement by the above methods and the fact that all the known L-asparaginases from *Escherichia coli* etc., other than mammal, exist in a tetrameric form, it can be estimated that the purified polypeptide exists in a tetrameric form. The method as used in Experiment 1-1(d) confirmed that the purified polypeptide has an L-asparaginase activity.

Example A-2(a)

Preparation of transformant

FIG.3 summarizes the procedures to prepare transformants. PCR was performed under the same conditions as used in Example A-1(a) except for the nucleotide sequences of a sense- and anti-sense-primers. As the sense- and anti-sense-primers, oligonucleotides with the nucleotide sequences of 5'-GTGAATTCGGAGGTTCAGAT-GGCGCGCGCATCA-3' and 5'-CTGCGGCCGCTCAGATGGCAGGCGGCAC-3' were respectively used. The DNA thus amplified was cleaved by restriction enzymes *Eco* RI and *Not I* to obtain an about 1.7 kbp *Eco RI-Not* I fragment. Seventy ng of the DNA fragment was mixed with 50 ng of a plasmid vector, "pBPV", commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, cleaved in advance by restriction enzymes *Xho* I and *Not* I, and 25 ng of each of 4 oligonucleotides as linkers with nucleotide sequences of 5'-TCGAGCCACCATGAAGTGTTCGTGGGTTATT-3', 5-TTCTTCCTGATGGCCGTAGTGACAGGAGTG-3', 5'-AATTCACTCCTGTCACTACGGCCATCAGGA-3',and 5'-AGAAAATAACCCACGAACACTTCATGGTGGC-3'. The oligonucleotides for linkers were synthesized in a usual manner and used after reacted with T4 polynucleotide kinase, commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, and purified by ethanol-precipitation. To the DNA mixture was added the solution I in "LIGATION KIT VERSION 2", commercialized by Takara Shuzo, Tokyo, Japan. The mixture was incubated at 16°C for 2 hours to obtain a replicable recombinant DNA "pBIgGPA/WT".

The recombinant DNA pBIgGPA/WT was introduced into an *Escherichia coli* HB101 strain by the competent cell method. The transformant thus obtained was inoculated into L broth medium (pH 7.2) containing 50 μg/ml ampicillin followed by cultivation at 370C for 18 hours under shaking conditions. The transformants, collected by centrifuging the resulting culture, were subjected to a conventional alkali-SDS method to extract the recombinant DNA pBIgGPA/WT. The nucleotide sequence analysis using an automatic sequencer confirmed that the recombinant DNA pBIgGPA/WT had the structure in FIG.4: A DNA encoding a polypeptide containing a signal sequence for immunoglobulin secretion, as shown by D. F. Stern et al. in *"Science"*, Vol.235, pp.321-324 (1984), i.e., "Ig sec DNA" was ligated to the downstream of a region for transcriptional regulation, comprising an enhancer derived from long terminal repeats of Moloney Mouse Sarcoma Virus (Emsv), and a promotor derived from Mouse metallothionein I gene (Pmti). Furthermore, GPA/WT DNA was ligated in the same frame to the downstream of the Ig sec DNA in the direction from the 5'- to 3'-termini of GPA/WT DNA. It was also confirmed that a termination codon exists in the 3'-terminus of GPA/WT DNA without intervening

sequences.

The recombinant DNA pBIgGPA/WT was introduced into a cell line C127 (ATCC CRL-1616), derived from a mouse, by using a lipofectin® reagent commercialized by Life Technologies, Inc., Gaitherburg, U.S.A., according to the attached protocol. The transformants with the recombinant DNA were selected based on the lack of proliferation-regulatory ability, i.e., focus-forming ability, as a first selection. The cells around those containing foci were collected using sterilized filter papers and subjected to a conventional limiting dilution method to form single cells which were then selected depending on the productivity of L-asparaginase, as final selection. Thus, a transformant, "C-GPA/WT", was obtained.

Eample A-2(b)

Production of polypeptide

The transformant C-GPA/WT was inoculated into a well of "3046", a plastic multiwell plate with 6 wells, 3.5 cm in diameter, commercialized by Becton Dickinson Labware, New Jersey, U.S.A., with DME medium containing 10 v/v % bovine fetal serum, and cultured to be confluent as a seed culture. Some of the cells, scraped by treatment with trypsin, were inoculated as seed cells into each of the multiwell plates which were charged with the fresh preparation of the same medium and cultured. After repeating manipulations similarly as in the above and with scale up to increase the cell number, the cells were subjected to a conventional continuous culture using 50 of 150 cm^2 culture flasks. The resulting culture supernatants of a volume of 100 1 was collected and treated with similar methods for treating the supernatant from the cell-disruptants in Example A-1(b): salting out with ammonium sulfate, the chromatography of the solution of the precipitates using Q SEPHAROSE FF COLUMN, the chromatography of the eluted fractions using L-ASPARAGINE AGAROSE, and the chromatography of the eluted fractions using HILOAD SUPERDEX 200 COLUMN. Consequently, a purified polypeptide with a purity of 90 % or more was obtained in a yield of about one µg/ml-culture.

Example A-2(c)

Physicochemical property of polypeptide

By testing similarly as in Example A-1(c), it was confirmed that the purified polypeptide thus obtained had equivalent physicochemical properties with the that obtained in Example A-1(b).

Example A-3(a)

Preparation of transformant

PCRs were performed under the same conditions in Example A-1(a) except for the template and the sense- and anti-sense-primers. The DNA thus obtained were treated similarly as in Example A-1(a) to prepare recombinant DNAs, "pKGPA/D364stp", "pKHA/MUT1", "pKHA/MUT2", "pKHA/MUT3" and "pKHA/MUT5". Table 5 summarizes template DNAs and nucleotide sequences of a sense- and anti-sense-primers which were used to prepare the each recombinant DNAs. By sequencing similarly as in Example A-1(a), the structures of these recombinant DNAs were confirmed as shown in FIGs. 5 to 9.

Table 5

Recombinant DNA	Template DNA	Nucleotide sequences of sense (upper line) and anti-sense (lower line) primers *
pKGPA/D364stp	pCGPA/D364stp	5'-GCGAATTC*ATGGCGCGCGCATCA*-3' 5'-GCAAGCTTTCA*TGCCGTGGGCAGTGT*-3'
pKHA/MUT1	pCHA/MUT1	5'-GCGAATTC*ATGGCGCGCGCGGTG*-3' 5'-GCAAGCTTTCA*CACCGAGGGTGGCGT*-3'
pKHA/MUT2	pCHA/MUT2	the same as used for pKHA/MUT1 preparation the same as used for pKHA/MUT1 preparation
pKHA/MUT3	pCHA/MUT3	the same as used for pKHA/MUT1 preparation the same as used for pKHA/MUT1 preparation
pKHA/MUT5	pCHA/MUT5	the same as used for pKHA/MUT1 preparation the same as used for pKHA/MUT1 preparation

*) Italics in the upper line in each column mean the 5'-terminal nucleotide sequence of a DNA encoding L-asparaginase, and those in the lower line mean the complementary sequence to the 3'-terminus of the DNA, wherein the L-asparaginese originates from a guinea pig or human.

The recombinant DNAs were treated according to the methods as in Example A-1(a) to obtain transformants, "J-GPA/D364stp", "J-HA/MUT1", "J-HA/MUT2", "J-HA/MUT3" and "J-HA/MUTS".

Example A-3(b)

Production of polypeptide

The transformants obtained in Example A-3(a) were treated according to the methods similarly as in Example A-1(b): cultivation, disrupting the resulting cells, the precipitations of the cell-disruptants with ammonium sulfate, the chromatography of the precipitate solutions using Q SEPHAROSE FF COLUMN, and the chromatography of the eluted fractions using L-ASPARAGINE AGAROSE in that order. The eluted fractions thus obtained were concentrated using membranes similarly as in Example A-1(b) followed by subjecting the chromatography using HILOAD SUPERDEX 200 COLUMN to collect the eluted fractions with a molecular weight of about 140 kDa. Each system yielded the purified polypeptide with a purity of 90 % or more in a yield of about 0.1 mg/ml-culture. These purified polypeptides were analyzed by the methods as in Example A-1(c) to examine their physicochemical properties. Table 6 shows the results combined with those in Example A-1(c).

Table 6

Transformant, producing the polypeptide	Molecular weight (kDa) *1	Molecular weight (kDa) *2	L-asparaginase activity
J-GPA/WT	about 300	about 50 ± 10	+
J-GPA/D364stp	about 140	about 40	+
J-HA/MUT1	about 140	about 40	+
J-HA/MUT2	about 140	about 40	+
J-HA/MUT3	about 140	about 40	+
J-HA/MUT5	about 140	about 40	+

Note) The symbols "*1" and "*2" mean that the value was determined by gel filtration, and the value was determined by SDS-PAGE, respectively.

Table 6 indicates that all of the present polypeptides, expressed in *Escherichia coli* and purified, expressed an L-asparaginase activity. Furthermore, table 6 indicates the that the polypeptides formed tetramers.

Example A-4(a)

Preparation of transformants

PCRs were performed under the same conditions in Example A-1(a) except for the template and the sense- and anti-sense-primers. DNAs thus obtained were ligated with the same linkers as used in Example A-2(a) under the same conditions as in Example A-2(a) to obtain recombinant DNAs, "pBIgGPA/D364stp", "pBIgHA/MUT1", "pBIgHA/MUT2", "pBIgHA/MUT3" and "pBIgHA/MUT5". Table 7 summarizes template DNAs and nucleotide sequences of sense- and anti-sense-primers which were used to prepare the each recombinant DNAs. By sequencing similarly as in Example A-1(a), the structures of these recombinant DNAs were confirmed as shown in FIGs.10 to 14.

Table 7

Recombinant DNA	Template DNA	Nucleotide sequences of sense (upper line) and anti-sense (lower line) primers *
pBIgGPA/D364stp	pCGPA/D364stp	5'-GTGAATTCGGAGGTTCAG*ATGGCGCGCGCATCA*-3' 5'-CTGCGGCCGCTCA*TGCCGTGGGCAGTG*-3'
pBIgHA/MUT1	pCHA/MUT1	5'-CTGAATTCGGAGGTTCAG*ATGGCGCGCGCGGTG*-3' 5'-CTGCGGCCGCTCA*CACCGAGGGTGGCG*-3'
pBIgHA/MUT2	pCHA/MUT2	the same as used for pBIgHA/MUT1 preparation the same as used for pBIgHA/MUT1 preparation
pBIgHA/MUT3	pCHA/MUT3	the same as used for pBIgHA/MUT1 preparation the same as used for pBIgHA/MUT1 preparation
pBIgHA/MUT5	pCHA/MUT5	the same as used for pBIgHA/MUT1 preparation the same as used for pBIgHA/MUT1 preparation

Note) *: Italics in the upper line in each column mean the 5'-terminal nucleotide sequence of a DNA encoding L-asparaginase, and those in the lower line mean the complementary sequence to the 3'-terminus of the DNA, wherein the L-asparaginese originates from a guinea pig or human.

The recombinant DNAs thus obtained were treated similarly as in Example A-2(a) to obtain transformants, "C-GPA/D364stp", "C-HA/MUT1", "C-HA/MUT2", "C-HA/MUT3" and "C-HA/MUTS".

Example A-4(b)

Production of polypeptide

The transformants obtained in Example A-4(a) were cultured according to the methods as in Example A-2(b), and the resulting culture supernatants were treated with similar methods for treating the supernatants from the cell-disruptants in Example A-1(b): the precipitations of culture supernatants with ammonium sulfate, the chromatography of the precipitate solutions using Q SEPHAROSE FF COLUMN, and the chromatography of the eluted fractions using L-ASPARAGINE AGAROSE in that order. The eluted fractions thus obtained were concentrated using membranes similarly as in Example A-1(b) followed by subjecting the chromatography using HILOAD SUPERDEX 200 COLUMN to collect the eluted fractions with a molecular weights of about 140 kDa. Each of these systems yielded the purified polypeptide with a purity of 90 % or more in a yield of about one μg/ml-culture. These purified polypeptides were analyzed by the methods as in Example A-1(c) to examine their physicochemical properties. Table 8 shows the results combined with those in Example A-3.

Table 8

The polypeptide-producing transformant	Molecular weight (kDa) *1	Molecular weight (kDa) *2	L-asparaginase activity
J-GPA/WT	about 300	about 50 ± 10	+
J-GPA/D364stp	about 140	about 40	+
J-HA/MUT1	about 140	about 40	+
J-HA/MUT2	about 140	about 40	+
J-HA/MUT3	about 140	about 40	+
J-HA/MUT5	about 140	about 40	+

Note) The symbols "*1" and "*2" mean that the value was determined by gel filtration, and the value was determined by SDS-PAGE, respectively.

Table 8 indicates that all of the present polypeptides, expressed in mammalian cells and purified, expressed an L-asparaginase activity. Furthermore, table 8 indicates the polypeptides formed tetramers.

As shown in above Example A, each of the polypeptides according to the present invention expresses an L-asparaginase activity. Therefore, the present agent for susceptive diseases hydrolyze L-asparagine in patients to exert therapeutic and preventive effects on L-asparaginase-susceptive diseases when administered to human. The wording "susceptive diseases" as referred in the present specification means diseases in general which are caused by the existence of tumor cells dependent on L-asparagine: For example, leukemias such as acute leukemia, an acute transformation of chronic leukemia and T-lymphocytic leukemia, and malignant tumors such as Hodgkin's diseases and non-Hodgkin's diseases. The present agent for susceptive diseases possesses thus the uses as anti-tumor agents for treating and/or preventing such susceptive diseases as above. Although it varies dependently on the types of agents used for such purposes and susceptive diseases to be treated, the present agent is generally processed into an agent in the form of a liquid, a paste or a solid which contains the polypeptides in an amount of 0.000001-100 w/w %, preferably, 0.0001-100 w/w %, on a dry solid basis.

The present agent can be used intact or processed into compositions by mixing with one or more selected from the group consisting of physiologically-acceptable carriers, excipients, solvents, buffers and stabilizers, and further, if necessary, other biologically-active substances and other agents. For example, "Iyakuhin-Tenkabutsu-Jiten (The Dictionary of Pharmaceutical Excipients)" (1994), edited by Japan Pharmaceutical Excipients Council, Tokyo, Japan, published by Yakujinippo LTD., Tokyo, Japan and "Iyakuhin-Tenkabutsu-Jiten-Tsuiho 1995 (Suppliment for The Dictionary of Pharmaceutical Excipients)" (1995), edited by Japan Pharmaceutical Excipients Council, Tokyo, Japan, published by Yakujinippo LTD., Tokyo, Japan, mention the embodiments of such carriers, excipients, solvents, buffers and stabilizers. Examples of such other biologically-active substances and other agents include interferon-α, interferon-β, interferon-γ, interleukin 1, interleukin 2, interleukin 3, TNF-a, TNF-β, GM-CSF, carboquone, cyclophosphamide, aclarbicin, thiotepa, busulfan, ancitabine, cytarabine, fluorouracil, 5-fluoro-1-(tetrahydro-2-furyl)uracil, methotrexate, actinomycin D, chromomycin A3, daunorubicin, doxorubicin, bleomycin, mercaptopurine, prednisolone, mitomycin C, vincristine, vinblastine, radio gold colloidal, Krestin®, picibanil, lentinan and Maruyama vaccine.

The present agent for susceptive diseases includes those in a unit dose form which means a physically separated and formed medicament suitable for administration, and contains the polypeptides in a daily dose or in a dose from 1/40 to several folds (up to 4 folds) of the daily dose. Examples of such medicaments are injections, liquids, powders, granules, tablets, capsules, sublinguals, ophthalmic solutions, nasal drops and suppositories.

The present agent can be administered to patients orally or parenterally. In both administrations, the agent exerts a satisfactory effect in the treatment and/or the prevention for the susceptive diseases. Although it varies dependently on the types of susceptive diseases and their symptoms, the agent can be orally administered to patients or parenterally administered to patients' intradermal tissues, subcutaneous tissues, muscles, and veins at a dose as amounts of the polypeptides in the range of about 0.1 μg - 500 mg/shot, preferably, about 0.1-100 mg/shot, 1-4 times/day or 1-7 times/week, for one day to one year. The present agent for susceptive diseases further includes the forms by applying gene therapy. When a transformant into which the DNAs encoding the polypeptides of this invention are introduced are administered to patients to express in them, they exert equivalent effects as above administrations. For example, "Jikken-Igaku Bessatsu, Bio-manual Up Series, Idenshi-Chiryo-No-Kisogijutsu (Basic Techniques for Gene Therapy)" (1996), edited by Takashi SHIMADA, Izumi SAITO and Takaya OZAWA, published by Yodosha, Tokyo, Japan, details the general procedures for the gene therapy.

The biological activities and acute toxicity of the present polypeptides are explained based on Experiment 3 and 4 below, respectively.

Experiment 3

Biological activity

Experiment 3-1

Antitumor effect *in vitro*

A human histocytic lymphoma cell line U937 (ATCC CRL-1593), and a cell line Molt4 (ATCC CRL-1582), derived from human T lymphoblasts, were subcultured in RPMI 1640 medium containing 10 v/v % bovine fetal serum. The cells collected by centrifugation from each subculturing system in logarithmic phase were suspended in the same medium to give a concentration of 2 x 10^5 cells/ml. Every one ml of the each cell suspension was charged into each of 13 wells of multiwell plates with 24 wells, "3047", commercialized by Becton Dickinson Labware, New Jersey, U.S. A. After each of dilutions of 12 types of the purified polypeptides prepared in Example A-1 to A-4 with PBS was further charged into the each well, the cells were cultured at 37°C for 72 hours in a 5 v/v % CO_2 incubator. The final concen-

tration of each of the purified polypeptides was one unit/ml as an L-asparaginase activity. As a control, after charged with equivalent volume of PBS, the cells were cultured correspondingly. The cells were collected after the cultivation to stain cells died with trypan blue. Cell survival ratio in each systems with the purified polypeptides was compared with that in the control. All of the cell survival ratios with the purified polypeptides were significantly lower than that in the control. These results indicate that all of the present polypeptides, obtained in Examples A-1 to A-4, have cytotoxicity to U937 and Molt4.

Experiment 3-2

Antitumor effect *in vivo*

For model mice were used C3H mice wherein a mouse lymphoma cell line 6C3HED, registered in Cell Resource Center for Biomedical Research, Institute of Development Aging and Cancer, Tohoku University, Sendai, Japan, was transplanted with passages by subcutaneous injections at their sides in a range of 1×10^7 cells/body every 8 days in usual manner. To the model mice were administered the purified polypeptides obtained in Example A-1 to A-4 in the range of 400 unit/body by venoclyses every day from fourth to seventh days after transplanted with the cells. Dimensions of the tumors were observed with naked eyes on fourth and eighth day after the transplantations. The purified polypeptides were administered after diluted with 0.15 M NaCl and filtrated with membrane filters, 0.45 μm in pore size, commercialized by Millipore Corp., Bedford, U.S.A. As a control, 0.15 M NaCl was treated correspondingly. While significant enlargements of the tumors were observed in the control, significant involutions or disappearances of the tumors were observed in mice administered with the polypeptides. These results indicates that all of the present polypeptides, obtained in Examples A-1 to A-4, are able to cure the tumors of model mice.

Experiment 4

Acute toxicity

The purified polypeptides obtained in Examples A-1 to A-4 were separately administered to 8-week-old mice percutaneously, perorally or intraperitoneally according to conventional manner. The LD_{50} of all the polypeptides was about 100 mg/kg or higher independently of the administration routes. These results evidenced that the present polypeptides could be safely incorporated into pharmaceuticals for administering human.

The following examples explain the present agent for susceptive diseases.

Example B-1

Solution

The purified polypeptides obtained in Examples A-1 to A-4 were separately dissolved to give a concentration of 0.1 mg/ml in physiological saline containing one w/v % human serum albumin as a stabilizer, and sterilized with membrane filters according to conventional manner to obtain solutions.

All of the products have satisfactory stabilities and can be used as injections, ophthalmic solutions, collunarium in the treatment and/or the prevention of susceptive diseases including a malignant tumor, acute leukemia, malignant lymphoma, an acute transformation of chronic leukemia, T-lymphocytic leukemia.

Example B-2

Solution

The purified polypeptides obtained in Examples A-1 to A-4 were separately dissolved to give a concentration of 0.1 mg/ml in physiological saline containing one w/v % glycerol as a stabilizer, and sterilized with membrane filters according to conventional manner to obtain solutions.

All of the products have satisfactory stabilities and can be used as injections, ophthalmic solutions, collunarium for the treatment and/or the prevention of susceptive diseases including a malignant tumor, acute leukemia, malignant lymphoma, an acute transformation of chronic leukemia and T-lymphocytic leukemia.

Example B-3

Dry injection

The purified polypeptides obtained in Examples A-1 to A-4 were separately dissolved to give a concentration of 50 mg/ml in physiological saline containing one w/v % purified gelatin as a stabilizer, and the solutions were sterilized with membrane filters according to conventional manner. One ml aliquots of the sterilized solutions were distributed to vials, lyophilized and cap sealed.

All of the products have satisfactory stabilities and can be used as dry injections for the treatment and/or the prevention of susceptive diseases including a malignant tumor, acute leukemia, malignant lymphoma, an acute transformation of chronic leukemia and T-lymphocytic leukemia.

Example B-4

Ointment

"HI-BIS-WAKO 104", a carboxyvinyl polymer commercialized by Wako Pure Chemicals, Tokyo, Japan, and a purified trehalose were dissolved in sterilized distilled water to give concentrations of 1.4 w/w % and 2.0 w/w %, respectively, and the purified polypeptides obtained in Examples A-1 to A-4 were separately mixed to homogeneity in the solutions followed by adjusting the pH of the resulting solutions to pH 7.2 to obtain pastes containing about one mg/g of the polypeptides.

All of the products have satisfactory spreadabilities and stabilities, and can be used as ointments for treating and/or preventing susceptive diseases including a malignant tumor, acute leukemia, malignant lymphoma, an acute transformation of chronic leukemia and T-lymphocytic leukemia.

Example B-5

Tablet

Any one of the purified polypeptides obtained in Examples A-1 to A-4 and LUMIN, i.e. [bis-4-(1-ethylquinoline)][γ-4'-(1-ethylquinoline] pentamethionine cyanine, as a cell activator were mixed to homogeneity with "FINETOSE®" an hydrous crystalline α-maltose commercialized by Hayashibara Co.,Ltd., Okayama, Japan, and the mixtures were tabletted by tabletting machine to obtain tablets, about 200 mg weight each, containing the polypeptide and the LUMIN, about 5 mg each.

All of the products have satisfactory swallowing abilities, stabilities and cell activating activities, and can be used for treating and/or preventing susceptive diseases including a malignant tumor, acute leukemia, malignant lymphoma, an acute transformation of chronic leukemia and T-lymphocytic leukemia.

The present invention is based on the findings of polypeptides which originate from mammal, having L-asparaginase activity. The polypeptides are substances which have revealed amino acid sequences totally, and stable activities to hydrolyze L-asparagine. Therefore, the polypeptides exert satisfactory effects in the treatment and/or the prevention for diseases caused by tumor cells dependent on L-asparagine.

The polypeptides originate from mammal, so that they have low antigenicities to human and don't cause serious side effects even when administered in large amounts or continuously. Therefore, the polypeptides have the advantage that they can exert desired effects without restricted controls on patients' sensitivities.

The polypeptides thus valuable can be produced in desired amounts using the present DNAs encoding them.

Thus, the present invention is a significant invention which has a remarkable effect and gives a great contribution to this field.

While there has been described what is at present considered to be the preferred embodiments of the present invention, it will be understood the various modifications may be made therein, and it is intended to cover in the appended claims all such modifications as fall within the true spirits and scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
NAME:KABUSHIKI KAISHA HAYASHIBARA SEIBUTSU KAGAKU KENKYUJO

(ii) TITLE OF INVENTION:POLYPEPTIDES HAVING L-ASPARAGINASE ACTIVITY

(iii) NUMBER OF SEQUENCES:18

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(F) POSTAL CODE (ZIP):700

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE:Floppy disk
(B) COMPUTER:IBM PC compatible
(C) OPERATING SYSTEM:PC-DOS/MS-DOS
(D) SOFTWARE:Word Perect Version 5.1

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:JP 168,172/96
(B) FILING DATE:June 7, 1996

(2)INFORMATION FOR SEQ ID NO:1:
(i)SEQUENCE CHARACTERISTICS:
(A)LENGTH:4 amino acids
(B)TYPE:amino acid
(D)TOPOLOGY:linear
(ii)MOLECULE TYPE:peptide
(xi)SEQUENCE DESCRIPTION:SEQ ID NO:1:

Thr Gly Gly Thr
1

(3)INFORMATION FOR SEQ ID NO:2:
(i)SEQUENCE CHARACTERISTICS:
(A)LENGTH:5 amino acids
(B)TYPE:amino acid
(D)TOPOLOGY:linear
(ii)MOLECULAR TYPE:peptide
(xi)SEQUENCE DESCRIPTION:SEQ ID NO:2:

His Gly Thr Asp Thr
1 5

(4) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:5 amino acids
(B) TYPE:amino acid
(D) TOPOLOGY:linear
(ii) MOLECULAR TYPE:peptide
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:3:

Gln Cys Leu Xaa Gly
1 5

(5) INFORMATION FOR SEQ ID NO:4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:363 amino acids
(B) TYPE:amino acid
(D) TOPOLOGY:linear
(ii) MOLECULAR TYPE:peptide
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:4:

Met Ala Arg Ala Ser Gly Ser Glu Arg His Leu Leu Leu Ile Tyr Thr
1 5 10 15
Gly Gly Thr Leu Gly Met Gln Ser Lys Gly Gly Val Leu Val Pro Gly
20 25 30
Pro Gly Leu Val Thr Leu Leu Arg Thr Leu Pro Met Phe His Asp Lys
35 40 45
Glu Phe Ala Gln Ala Gln Gly Leu Pro Asp His Ala Leu Ala Leu Pro
50 55 60
Pro Ala Ser His Gly Pro Arg Val Leu Tyr Thr Val Leu Glu Cys Gln
65 70 75 80
Pro Leu Leu Asp Ser Ser Asp Met Thr Ile Asp Asp Trp Ile Arg Ile
85 90 95
Ala Lys Ile Ile Glu Arg His Tyr Glu Gln Tyr Gln Gly Phe Val Val
100 105 110
Ile His Gly Thr Asp Thr Met Ala Phe Gly Ala Ser Met Leu Ser Phe
115 120 125
Met Leu Glu Asn Leu His Lys Pro Val Ile Leu Thr Gly Ala Gln Val
130 135 140
Pro Ile Arg Val Leu Trp Asn Asp Ala Arg Glu Asn Leu Leu Gly Ala
145 150 155 160
Leu Leu Val Ala Gly Gln Tyr Ile Ile Pro Glu Val Cys Leu Phe Met
165 170 175
Asn Ser Gln Leu Phe Arg Gly Asn Arg Val Thr Lys Val Asp Ser Gln
180 185 190
Lys Phe Glu Ala Phe Cys Ser Pro Asn Leu Ser Pro Leu Ala Thr Val
195 200 205
Gly Ala Asp Val Thr Ile Ala Trp Asp Leu Val Arg Lys Val Asn Trp
210 215 220
Lys Asp Pro Leu Val Val His Ser Asn Met Glu His Asp Val Ala Leu
225 230 235 240
Leu Arg Leu Tyr Pro Gly Ile Pro Ala Ser Leu Val Arg Ala Phe Leu

```
                    245                 250                 255
Gln Pro Pro Leu Lys Gly Val Val Leu Glu Thr Phe Gly Ser Gly Asn
                260                 265                 270
Gly Pro Ser Lys Pro Asp Leu Leu Gln Glu Leu Arg Ala Ala Ala Gln
            275                 280                 285
Arg Gly Leu Ile Met Val Asn Cys Ser Gln Cys Leu Arg Gly Ser Val
        290                 295                 300
Thr Pro Gly Tyr Ala Thr Ser Leu Ala Gly Ala Asn Ile Val Ser Gly
305                 310                 315                 320
Leu Asp Met Thr Ser Glu Ala Ala Leu Ala Lys Leu Ser Tyr Val Leu
                325                 330                 335
Gly Leu Pro Glu Leu Ser Leu Glu Arg Arg Gln Glu Leu Leu Ala Lys
            340                 345                 350
Asp Leu Arg Gly Glu Met Thr Leu Pro Thr Ala
        355                 360         363
```

(6) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:565 amino acids

(B) TYPE:amino acid

(D) TOPOLOGY:linear

(ii) MOLECULAR TYPE:peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:5:

```
Met Ala Arg Ala Ser Gly Ser Glu Arg His Leu Leu Leu Ile Tyr Thr
1               5                   10                  15
Gly Gly Thr Leu Gly Met Gln Ser Lys Gly Gly Val Leu Val Pro Gly
            20                  25                  30
Pro Gly Leu Val Thr Leu Leu Arg Thr Leu Pro Met Phe His Asp Lys
        35                  40                  45
Glu Phe Ala Gln Ala Gln Gly Leu Pro Asp His Ala Leu Ala Leu Pro
    50                  55                  60
Pro Ala Ser His Gly Pro Arg Val Leu Tyr Thr Val Leu Glu Cys Gln
65                  70                  75                  80
Pro Leu Leu Asp Ser Ser Asp Met Thr Ile Asp Asp Trp Ile Arg Ile
                85                  90                  95
Ala Lys Ile Ile Glu Arg His Tyr Glu Gln Tyr Gln Gly Phe Val Val
            100                 105                 110
Ile His Gly Thr Asp Thr Met Ala Phe Gly Ala Ser Met Leu Ser Phe
        115                 120                 125
Met Leu Glu Asn Leu His Lys Pro Val Ile Leu Thr Gly Ala Gln Val
    130                 135                 140
Pro Ile Arg Val Leu Trp Asn Asp Ala Arg Glu Asn Leu Leu Gly Ala
145                 150                 155                 160
Leu Leu Val Ala Gly Gln Tyr Ile Ile Pro Glu Val Cys Leu Phe Met
                165                 170                 175
Asn Ser Gln Leu Phe Arg Gly Asn Arg Val Thr Lys Val Asp Ser Gln
            180                 185                 190
Lys Phe Glu Ala Phe Cys Ser Pro Asn Leu Ser Pro Leu Ala Thr Val
        195                 200                 205
Gly Ala Asp Val Thr Ile Ala Trp Asp Leu Val Arg Lys Val Asn Trp
    210                 215                 220
Lys Asp Pro Leu Val Val His Ser Asn Met Glu His Asp Val Ala Leu
225                 230                 235                 240
Leu Arg Leu Tyr Pro Gly Ile Pro Ala Ser Leu Val Arg Ala Phe Leu
```

```
                    245                 250                 255
Gln Pro Pro Leu Lys Gly Val Val Leu Glu Thr Phe Gly Ser Gly Asn
                260                 265                 270
Gly Pro Ser Lys Pro Asp Leu Leu Gln Glu Leu Arg Ala Ala Ala Gln
            275                 280                 285
Arg Gly Leu Ile Met Val Asn Cys Ser Gln Cys Leu Arg Gly Ser Val
        290                 295                 300
Thr Pro Gly Tyr Ala Thr Ser Leu Ala Gly Ala Asn Ile Val Ser Gly
305                 310                 315                 320
Leu Asp Met Thr Ser Glu Ala Ala Leu Ala Lys Leu Ser Tyr Val Leu
                325                 330                 335
Gly Leu Pro Glu Leu Ser Leu Glu Arg Arg Gln Glu Leu Leu Ala Lys
            340                 345                 350
Asp Leu Arg Gly Glu Met Thr Leu Pro Thr Ala Asp Leu His Gln Ser
        355                 360                 365
Ser Pro Pro Gly Ser Thr Leu Gly Gln Gly Val Ala Arg Leu Phe Ser
    370                 375                 380
Leu Phe Gly Cys Gln Glu Glu Asp Ser Val Gln Asp Ala Val Met Pro
385                 390                 395                 400
Ser Leu Ala Leu Ala Leu Ala His Ala Gly Glu Leu Glu Ala Leu Gln
                405                 410                 415
Ala Leu Met Glu Leu Gly Ser Asp Leu Arg Leu Lys Asp Ser Asn Gly
            420                 425                 430
Gln Thr Leu Leu His Val Ala Ala Arg Asn Gly Arg Asp Gly Val Val
        435                 440                 445
Thr Met Leu Leu His Arg Gly Met Asp Val Asn Ala Arg Asp Arg Asp
    450                 455                 460
Gly Leu Ser Pro Leu Leu Leu Ala Val Gln Gly Arg His Arg Glu Cys
465                 470                 475                 480
Ile Arg Leu Leu Arg Lys Ala Gly Ala Cys Leu Ser Pro Gln Asp Leu
                485                 490                 495
Lys Asp Ala Gly Thr Glu Leu Cys Arg Leu Ala Ser Arg Ala Asp Met
            500                 505                 510
Glu Gly Leu Gln Ala Trp Gly Gln Ala Gly Ala Asp Leu Gln Gln Pro
        515                 520                 525
Gly Tyr Asp Gly Arg Ser Ala Leu Cys Val Ala Glu Ala Ala Gly Asn
    530                 535                 540
Gln Glu Val Leu Ala Leu Leu Arg Asn Leu Ala Leu Val Gly Pro Glu
545                 550                 555                 560
Val Pro Pro Ala Ile
                565
```

(7) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:365 amino acids

(B) TYPE:amino acid

(D) TOPOLOGY:linear

(ii) MOLECULAR TYPE:peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:6:

```
Met Ala Arg Ala Val Gly Pro Glu Arg Arg Leu Leu Ala Val Tyr Thr
1               5                   10                  15
Gly Gly Thr Ile Gly Met Arg Ser Glu Leu Gly Val Leu Val Pro Gly
            20                  25                  30
Thr Gly Leu Ala Ala Ile Leu Arg Thr Leu Pro Met Phe His Asp Glu
```

```
          35                  40                  45
Glu His Ala Arg Ala Arg Gly Leu Ser Glu Asp Thr Leu Val Leu Pro
      50                  55                  60
Pro Asp Ser Arg Asn Gln Arg Ile Leu Tyr Thr Val Leu Glu Cys Gln
65                  70                  75                  80
Pro Leu Phe Asp Ser Ser Asp Met Thr Ile Ala Glu Trp Val Arg Val
                85                  90                  95
Ala Gln Thr Ile Lys Arg His Tyr Glu Gln Tyr His Gly Phe Val Val
            100                 105                 110
Ile His Gly Thr Asp Thr Met Ala Phe Ala Ala Ser Met Leu Ser Phe
        115                 120                 125
Met Leu Glu Asn Leu Gln Lys Thr Val Ile Leu Thr Gly Ala Gln Val
    130                 135                 140
Pro Ile His Ala Leu Trp Ser Asp Gly Arg Glu Asn Leu Leu Gly Ala
145                 150                 155                 160
Leu Leu Met Ala Gly Gln Tyr Val Ile Pro Glu Val Cys Leu Phe Phe
                165                 170                 175
Gln Asn Gln Leu Phe Arg Gly Asn Arg Ala Thr Lys Val Asp Ala Arg
            180                 185                 190
Arg Phe Ala Ala Phe Cys Ser Pro Asn Leu Leu Pro Leu Ala Thr Val
        195                 200                 205
Gly Ala Asp Ile Thr Ile Asn Arg Glu Leu Val Arg Lys Val Asp Gly
    210                 215                 220
Lys Ala Gly Leu Val Val His Ser Ser Met Glu Gln Asp Val Gly Leu
225                 230                 235                 240
Leu Arg Leu Tyr Pro Gly Ile Pro Ala Ala Leu Val Arg Ala Phe Leu
                245                 250                 255
Gln Pro Pro Leu Lys Gly Val Val Met Glu Thr Phe Gly Ser Gly Asn
            260                 265                 270
Gly Pro Thr Lys Pro Asp Leu Leu Gln Glu Leu Arg Val Ala Thr Glu
        275                 280                 285
Arg Gly Leu Val Ile Val Asn Cys Thr Gln Cys Leu Arg Gly Ala Val
    290                 295                 300
Thr Thr Asp Tyr Ala Ala Gly Met Ala Met Ala Gly Ala Asn Val Ile
305                 310                 315                 320
Ser Gly Phe Asp Met Thr Ser Glu Ala Ala Leu Ala Lys Leu Ser Tyr
                325                 330                 335
Val Leu Gly Gln Pro Gly Leu Ser Leu Asp Val Arg Lys Glu Leu Leu
            340                 345                 350
Thr Lys Asp Leu Arg Gly Glu Met Thr Pro Pro Ser Val
        355                 360                 365
```

(8) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:365 amino acids

(B) TYPE:amino acid

(D) TOPOLOGY:linear

(ii) MOLECULAR TYPE:peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:7:

```
Met Ala Arg Ala Val Gly Pro Glu Arg Arg Leu Leu Ala Val Tyr Thr
1               5                  10                  15
Gly Gly Thr Ile Gly Met Arg Ser Glu Leu Gly Val Leu Val Pro Gly
            20                  25                  30
Thr Gly Leu Ala Ala Ile Leu Arg Thr Leu Pro Met Phe His Asp Glu
```

```
          35                  40                  45
Glu His Ala Arg Ala Arg Gly Leu Ser Glu Asp Thr Leu Val Leu Pro
    50                  55                  60
Pro Asp Ser Arg Asn Gln Arg Ile Leu Tyr Thr Val Leu Glu Cys Gln
65                  70                  75                  80
Pro Leu Phe Asp Ser Ser Asp Met Thr Ile Ala Glu Trp Val Arg Val
                85                  90                  95
Ala Gln Thr Ile Lys Arg His Tyr Glu Gln Tyr His Gly Phe Val Val
            100                 105                 110
Ile His Gly Thr Asp Thr Met Ala Phe Ala Ala Ser Met Leu Ser Phe
        115                 120                 125
Met Leu Glu Asn Leu Gln Lys Thr Val Ile Leu Thr Gly Ala Gln Val
    130                 135                 140
Pro Ile His Ala Leu Trp Ser Asp Gly Arg Glu Asn Leu Leu Gly Ala
145                 150                 155                 160
Leu Leu Met Ala Gly Gln Tyr Val Ile Pro Glu Val Cys Leu Phe Phe
                165                 170                 175
Gln Asn Gln Leu Phe Arg Gly Asn Arg Ala Thr Lys Val Asp Ala Arg
            180                 185                 190
Arg Phe Ala Ala Phe Cys Ser Pro Asn Leu Leu Pro Leu Ala Thr Val
        195                 200                 205
Gly Ala Asp Ile Thr Ile Asn Arg Glu Leu Val Arg Lys Val Asp Gly
    210                 215                 220
Lys Ala Gly Leu Val Val His Ser Ser Met Glu Gln Asp Val Gly Leu
225                 230                 235                 240
Leu Arg Leu Tyr Pro Gly Ile Pro Ala Ala Leu Val Arg Ala Phe Leu
                245                 250                 255
Gln Pro Pro Leu Lys Gly Val Val Met Glu Thr Phe Gly Ser Gly Asn
            260                 265                 270
Gly Pro Thr Lys Pro Asp Leu Leu Gln Glu Leu Arg Val Ala Thr Glu
        275                 280                 285
Arg Gly Leu Val Ile Val Asn Cys Thr Gln Cys Leu Arg Gly Ala Val
    290                 295                 300
Thr Thr Asp Tyr Ala Ala Gly Met Ala Met Ala Gly Ala Gly Val Ile
305                 310                 315                 320
Ser Gly Phe Asp Met Thr Ser Glu Ala Ala Leu Ala Lys Leu Ser Tyr
                325                 330                 335
Val Leu Gly Gln Pro Gly Leu Ser Leu Asp Val Arg Lys Glu Leu Leu
            340                 345                 350
Thr Lys Asp Leu Arg Gly Glu Met Thr Pro Pro Ser Val
        355                 360                 365
```

(9) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:365 amino acids

(B) TYPE:amino acid

(D) TOPOLOGY:linear

(ii) MOLECULAR TYPE:peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:8:

```
Met Ala Arg Ala Val Gly Pro Glu Arg Arg Leu Leu Ala Val Tyr Thr
1               5                   10                  15
Gly Gly Thr Ile Gly Met Arg Ser Glu Leu Gly Val Leu Val Pro Gly
            20                  25                  30
Thr Gly Leu Ala Ala Ile Leu Arg Thr Leu Pro Met Phe His Asp Glu
```

```
            35                  40                  45
Glu His Ala Arg Ala Arg Gly Leu Ser Glu Asp Thr Leu Val Leu Pro
    50                  55                  60
Pro Asp Ser Arg Asn Gln Arg Ile Leu Tyr Thr Val Leu Glu Cys Gln
65                  70                  75                  80
Pro Leu Phe Asp Ser Ser Asp Met Thr Ile Ala Glu Trp Val Arg Val
                85                  90                  95
Ala Gln Thr Ile Lys Arg His Tyr Glu Gln Tyr His Gly Phe Val Val
            100                 105                 110
Ile His Gly Thr Asp Thr Met Ala Phe Ala Ala Ser Met Leu Ser Phe
        115                 120                 125
Met Leu Glu Asn Leu Gln Lys Thr Val Ile Leu Thr Gly Ala Gln Val
    130                 135                 140
Pro Ile His Ala Leu Trp Ser Asp Gly Arg Glu Asn Leu Leu Gly Ala
145                 150                 155                 160
Leu Leu Met Ala Gly Gln Tyr Val Ile Pro Glu Val Cys Leu Phe Phe
                165                 170                 175
Gln Asn Gln Leu Phe Arg Gly Asn Arg Ala Thr Lys Val Asp Ala Arg
            180                 185                 190
Arg Phe Ala Ala Phe Cys Ser Pro Asn Leu Leu Pro Leu Ala Thr Val
        195                 200                 205
Gly Ala Asp Ile Thr Ile Asn Arg Glu Leu Val Arg Lys Val Asp Gly
    210                 215                 220
Lys Ala Gly Leu Val Val His Ser Ser Met Glu Gln Asp Val Gly Leu
225                 230                 235                 240
Leu Arg Leu Tyr Pro Gly Ile Pro Ala Ala Leu Val Arg Ala Phe Leu
                245                 250                 255
Gln Pro Pro Leu Lys Gly Val Val Met Glu Thr Phe Gly Ser Gly Asn
            260                 265                 270
Gly Pro Thr Lys Pro Asp Leu Leu Gln Glu Leu Arg Val Ala Thr Glu
        275                 280                 285
Arg Gly Leu Val Ile Val Asn Cys Thr Gln Cys Leu Gln Gly Ala Val
    290                 295                 300
Thr Thr Asp Tyr Ala Ala Gly Met Ala Met Ala Gly Ala Asn Val Ile
305                 310                 315                 320
Ser Gly Phe Asp Met Thr Ser Glu Ala Ala Leu Ala Lys Leu Ser Tyr
                325                 330                 335
Val Leu Gly Gln Pro Gly Leu Ser Leu Asp Val Arg Lys Glu Leu Leu
            340                 345                 350
Thr Lys Asp Leu Arg Gly Glu Met Thr Pro Pro Ser Val
        355                 360                 365
```

(10) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:365 amino acids

(B) TYPE:amino acid

(D) TOPOLOGY:linear

(ii) MOLECULAR TYPE:peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:9:

```
Met Ala Arg Ala Val Gly Pro Glu Arg Arg Leu Leu Ala Val Tyr Thr
1               5                   10                  15
Gly Gly Thr Ile Gly Met Arg Ser Glu Leu Gly Val Leu Val Pro Gly
            20                  25                  30
Thr Gly Leu Ala Ala Ile Leu Arg Thr Leu Pro Met Phe His Asp Glu
```

```
          35                  40                  45
Glu His Ala Arg Ala Arg Gly Leu Ser Glu Asp Thr Leu Val Leu Pro
    50                  55                  60
Pro Asp Ser Arg Asn Gln Arg Ile Leu Tyr Thr Val Leu Glu Cys Gln
65                  70                  75                  80
Pro Leu Phe Asp Ser Ser Asp Met Thr Ile Ala Glu Trp Val Arg Val
                85                  90                  95
Ala Gln Thr Ile Lys Arg His Tyr Glu Gln Tyr His Gly Phe Val Val
            100                 105                 110
Ile His Gly Thr Asp Thr Met Ala Phe Ala Ala Ser Met Leu Ser Phe
        115                 120                 125
Met Leu Glu Asn Leu Gln Lys Thr Val Ile Leu Thr Gly Ala Gln Val
    130                 135                 140
Pro Ile His Ala Leu Trp Ser Asp Gly Arg Glu Asn Leu Leu Gly Ala
145                 150                 155                 160
Leu Leu Met Ala Gly Gln Tyr Val Ile Pro Glu Val Cys Leu Phe Phe
                165                 170                 175
Gln Asn Gln Leu Phe Arg Gly Asn Arg Ala Thr Lys Val Asp Ala Arg
            180                 185                 190
Arg Phe Ala Ala Phe Cys Ser Pro Asn Leu Leu Pro Leu Ala Thr Val
        195                 200                 205
Gly Ala Asp Ile Thr Ile Asn Arg Glu Leu Val Arg Lys Val Asp Gly
    210                 215                 220
Lys Ala Gly Leu Val Val His Ser Ser Met Glu Gln Asp Val Gly Leu
225                 230                 235                 240
Leu Arg Leu Tyr Pro Gly Ile Pro Ala Ala Leu Val Arg Ala Phe Leu
                245                 250                 255
Gln Pro Pro Leu Lys Gly Val Val Met Glu Thr Phe Gly Ser Gly Asn
            260                 265                 270
Gly Pro Thr Lys Pro Asp Leu Leu Gln Glu Leu Arg Val Ala Thr Glu
        275                 280                 285
Arg Gly Leu Val Ile Val Asn Cys Thr Gln Cys Leu Gln Gly Ala Val
    290                 295                 300
Thr Thr Asp Tyr Ala Ala Gly Met Ala Met Ala Gly Ala Gly Val Ile
305                 310                 315                 320
Ser Gly Phe Asp Met Thr Ser Glu Ala Ala Leu Ala Lys Leu Ser Tyr
                325                 330                 335
Val Leu Gly Gln Pro Gly Leu Ser Leu Asp Val Arg Lys Glu Leu Leu
            340                 345                 350
Thr Lys Asp Leu Arg Gly Glu Met Thr Pro Pro Ser Val
        355                 360                 365
```

(11) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:1089 base pairs

(B) TYPE:nucleic acid

(D) TOPOLOGY:linear

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:10:

```
ATGGCGCGCG CATCAGGCTC CGAGAGGCAC CTGCTGCTCA TCTACACTGG CGGCACTTTG   60
GGCATGCAGA GCAAGGGCGG GGTGCTCGTC CCCGGCCCAG GCCTGGTCAC TCTGCTGCGG  120
ACCCTGCCCA TGTTCCATGA CAAGGAGTTC GCCCAGGCCC AGGGCCTCCC TGACCATGCT  180
CTGGCGCTGC CCCCTGCCAG CCACGGCCCC AGGGTCCTCT ACACGGTGCT GGAGTGCCAG  240
CCCCTCTTGG ATTCCAGCGA CATGACCATC GATGATTGGA TTCGCATAGC CAAGATCATA  300
```

```
GAGAGGCACT ATGAGCAGTA CCAAGGCTTT GTGGTTATCC ACGGCACCGA CACCATGGCC   360
TTTGGGGCCT CCATGCTGTC CTTCATGCTG GAAAACCTGC ACAAACCAGT CATCCTCACT   420
GGCGCCCAGG TGCCAATCCG TGTGCTGTGG AATGACGCCC GGGAAAACCT GCTGGGGGCG   480
TTGCTTGTGG CCGGCCAATA CATCATCCCT GAGGTCTGCC TGTTTATGAA CAGTCAGCTG   540
TTTCGGGGAA ACCGGGTAAC CAAGGTGGAC TCCCAGAAGT TTGAGGCCTT CTGCTCCCCC   600
AATCTGTCCC CACTAGCCAC TGTGGGCGCG GATGTCACAA TTGCCTGGGA CCTGGTGCGC   660
AAGGTCAACT GGAAGGACCC GCTGGTGGTG CACAGCAACA TGGAGCACGA CGTGGCACTG   720
CTGCGCCTCT ACCCTGGCAT CCCGGCCTCC CTGGTCCGGG CATTCCTGCA GCCCCCGCTC   780
AAGGGCGTGG TCCTGGAGAC CTTCGGCTCT GGCAACGGGC CGAGCAAGCC CGACCTGCTG   840
CAGGAGTTGC GGGCCGCGGC CCAGCGCGGC CTCATCATGG TCAACTGCAG CCAGTGCCTG   900
CGGGGGTCTG TGACCCCGGG CTATGCCACG AGCTTGGCGG GCGCCAACAT CGTGTCCGGC   960
TTAGACATGA CCTCAGAGGC CGCGCTGGCT AAGCTGTCCT ACGTGTTGGG CCTGCCGGAG  1020
CTGAGCCTGG AGCGCAGGCA GGAGCTGCTG GCCAAGGATC TTCGCGGGGA AATGACACTG  1080
CCCACGGCA                                                          1089
```

(12) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:1095 base pairs

(B) TYPE:nucleic acid

(D) TOPOLOGY:linear

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:11:

```
ATGGCGCGCG CGGTGGGGCC CGAGCGGAGG CTGCTGGCCG TCTACACCGG CGGCACCATT    60
GGCATGCGGA GTGAGCTCGG CGTGCTTGTG CCCGGGACGG GCCTGGCTGC CATCCTGAGG   120
ACACTGCCCA TGTTCCATGA CGAGGAGCAC GCCCGAGCCC GCGGCCTCTC TGAGGACACC   180
CTGGTGCTAC CCCCGGACAG CCGCAACCAG AGGATCCTCT ACACCGTGCT GGAGTGCCAG   240
CCCCTCTTCG ACTCCAGTGA CATGACCATC GCTGAGTGGG TTCGCGTTGC CCAGACCATC   300
AAGAGGCACT ACGAGCAGTA CCACGGCTTT GTGGTCATCC ACGGCACCGA CACCATGGCC   360
TTTGCTGCCT CGATGCTGTC CTTCATGCTG GAGAACCTGC AGAAGACTGT CATCCTCACT   420
GGGGCCCAGG TGCCCATCCA TGCCCTGTGG AGCGACGGCC GTGAGAACCT GCTGGGGGCA   480
CTGCTCATGG CTGGCCAGTA TGTGATCCCA GAGGTCTGCC TTTTCTTCCA GAATCAGCTG   540
TTTCGGGGCA ACCGGGCAAC CAAGGTAGAC GCTCGGAGGT TCGCAGCTTT CTGCTCCCCG   600
AACCTGCTGC CTCTGGCCAC AGTGGGTGCT GACATCACAA TCAACAGGGA GCTGGTGCGG   660
AAGGTGGACG GGAAGGCTGG GCTGGTGGTG CACAGCAGCA TGGAGCAGGA CGTGGGCCTG   720
CTGCGCCTCT ACCCTGGGAT CCCTGCCGCC CTGGTTCGGG CCTTCTTGCA GCCTCCCCTG   780
AAGGGCGTGG TCATGGAGAC CTTCGGTTCA GGGAACGGAC CCACCAAGCC CGACCTGCTG   840
CAGGAGCTGC GGGTGGCCAC CGAGCGCGGC CTGGTCATCG TCAACTGTAC CCAGTGCCTC   900
CGGGGGGCTG TGACCACAGA CTATGCAGCT GGCATGGCCA TGGCGGGAGC CAACGTCATC   960
TCAGGCTTCG ACATGACATC GGAGGCCGCC CTGGCCAAGC TATCGTATGT GCTGGGCCAG  1020
CCAGGGCTGA GCCTGGATGT CAGGAAGGAG CTGCTGACCA AGGACCTTCG GGGGGAGATG  1080
ACGCCACCCT CGGTG                                                   1095
```

(13) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:1095 base pairs

(B) TYPE:nucleic acid

(D) TOPOLOGY:linear

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:12:

```
ATGGCGCGCG CGGTGGGGCC CGAGCGGAGG CTGCTGGCCG TCTACACCGG CGGCACCATT    60
GGCATGCGGA GTGAGCTCGG CGTGCTTGTG CCCGGGACGG GCCTGGCTGC CATCCTGAGG   120
ACACTGCCCA TGTTCCATGA CGAGGAGCAC GCCCGAGCCC GCGGCCTCTC TGAGGACACC   180
```

```
CTGGTGCTAC CCCCGGACAG CCGCAACCAG AGGATCCTCT ACACCGTGCT GGAGTGCCAG  240
CCCCTCTTCG ACTCCAGTGA CATGACCATC GCTGAGTGGG TTCGCGTTGC CCAGACCATC  300
AAGAGGCACT ACGAGCAGTA CCACGGCTTT GTGGTCATCC ACGGCACCGA CACCATGGCC  360
TTTGCTGCCT CGATGCTGTC CTTCATGCTG GAGAACCTGC AGAAGACTGT CATCCTCACT  420
GGGGCCCAGG TGCCCATCCA TGCCCTGTGG AGCGACGGCC GTGAGAACCT GCTGGGGGCA  480
CTGCTCATGG CTGGCCAGTA TGTGATCCCA GAGGTCTGCC TTTTCTTCCA GAATCAGCTG  540
TTTCGGGGCA ACCGGGCAAC CAAGGTAGAC GCTCGGAGGT TCGCAGCTTT CTGCTCCCCG  600
AACCTGCTGC CTCTGGCCAC AGTGGGTGCT GACATCACAA TCAACAGGGA GCTGGTGCGG  660
AAGGTGGACG GGAAGGCTGG GCTGGTGGTG CACAGCAGCA TGGAGCAGGA CGTGGGCCTG  720
CTGCGCCTCT ACCCTGGGAT CCCTGCCGCC CTGGTTCGGG CCTTCTTGCA GCCTCCCCTG  780
AAGGGCGTGG TCATGGAGAC CTTCGGTTCA GGGAACGGAC CCACCAAGCC CGACCTGCTG  840
CAGGAGCTGC GGGTGGCCAC CGAGCGCGGC CTGGTCATCG TCAACTGTAC CCAGTGCCTC  900
CGGGGGGCTG TGACCACAGA CTATGCAGCT GGCATGGCCA TGGCGGGAGC CGGCGTCATC  960
TCAGGCTTCG ACATGACATC GGAGGCCGCC CTGGCCAAGC TATCGTATGT GCTGGGCCAG 1020
CCAGGGCTGA GCCTGGATGT CAGGAAGGAG CTGCTGACCA AGGACCTTCG GGGGGAGATG 1080
ACGCCACCCT CGGTG                                                   1095
```

(14) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:1095 base pairs

(B) TYPE:nucleic acid

(D) TOPOLOGY:linear

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:13:

```
ATGGCGCGCG CGGTGGGGCC CGAGCGGAGG CTGCTGGCCG TCTACACCGG CGGCACCATT   60
GGCATGCGGA GTGAGCTCGG CGTGCTTGTG CCCGGGACGG GCCTGGCTGC CATCCTGAGG  120
ACACTGCCCA TGTTCCATGA CGAGGAGCAC GCCCGAGCCC GCGGCCTCTC TGAGGACACC  180
CTGGTGCTAC CCCCGGACAG CCGCAACCAG AGGATCCTCT ACACCGTGCT GGAGTGCCAG  240
CCCCTCTTCG ACTCCAGTGA CATGACCATC GCTGAGTGGG TTCGCGTTGC CCAGACCATC  300
AAGAGGCACT ACGAGCAGTA CCACGGCTTT GTGGTCATCC ACGGCACCGA CACCATGGCC  360
TTTGCTGCCT CGATGCTGTC CTTCATGCTG GAGAACCTGC AGAAGACTGT CATCCTCACT  420
GGGGCCCAGG TGCCCATCCA TGCCCTGTGG AGCGACGGCC GTGAGAACCT GCTGGGGGCA  480
CTGCTCATGG CTGGCCAGTA TGTGATCCCA GAGGTCTGCC TTTTCTTCCA GAATCAGCTG  540
TTTCGGGGCA ACCGGGCAAC CAAGGTAGAC GCTCGGAGGT TCGCAGCTTT CTGCTCCCCG  600
AACCTGCTGC CTCTGGCCAC AGTGGGTGCT GACATCACAA TCAACAGGGA GCTGGTGCGG  660
AAGGTGGACG GGAAGGCTGG GCTGGTGGTG CACAGCAGCA TGGAGCAGGA CGTGGGCCTG  720
CTGCGCCTCT ACCCTGGGAT CCCTGCCGCC CTGGTTCGGG CCTTCTTGCA GCCTCCCCTG  780
AAGGGCGTGG TCATGGAGAC CTTCGGTTCA GGGAACGGAC CCACCAAGCC CGACCTGCTG  840
CAGGAGCTGC GGGTGGCCAC CGAGCGCGGC CTGGTCATCG TCAACTGTAC CCAGTGCCTC  900
CAGGGGGCTG TGACCACAGA CTATGCAGCT GGCATGGCCA TGGCGGGAGC CAACGTCATC  960
TCAGGCTTCG ACATGACATC GGAGGCCGCC CTGGCCAAGC TATCGTATGT GCTGGGCCAG 1020
CCAGGGCTGA GCCTGGATGT CAGGAAGGAG CTGCTGACCA AGGACCTTCG GGGGGAGATG 1080
ACGCCACCCT CGGTG                                                   1095
```

(15) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:1095 base pairs

(B) TYPE:nucleic acid

(D) TOPOLOGY:linear

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:14:

```
ATGGCGCGCG CGGTGGGGCC CGAGCGGAGG CTGCTGGCCG TCTACACCGG CGGCACCATT   60
GGCATGCGGA GTGAGCTCGG CGTGCTTGTG CCCGGGACGG GCCTGGCTGC CATCCTGAGG  120
```

```
ACACTGCCCA TGTTCCATGA CGAGGAGCAC GCCCGAGCCC GCGGCCTCTC TGAGGACACC  180
CTGGTGCTAC CCCCGGACAG CCGCAACCAG AGGATCCTCT ACACCGTGCT GGAGTGCCAG  240
CCCCTCTTCG ACTCCAGTGA CATGACCATC GCTGAGTGGG TTCGCGTTGC CCAGACCATC  300
AAGAGGCACT ACGAGCAGTA CCACGGCTTT GTGGTCATCC ACGGCACCGA CACCATGGCC  360
TTTGCTGCCT CGATGCTGTC CTTCATGCTG GAGAACCTGC AGAAGACTGT CATCCTCACT  420
GGGGCCCAGG TGCCCATCCA TGCCCTGTGG AGCGACGGCC GTGAGAACCT GCTGGGGGCA  480
CTGCTCATGG CTGGCCAGTA TGTGATCCCA GAGGTCTGCC TTTTCTTCCA GAATCAGCTG  540
TTTCGGGGCA ACCGGGCAAC CAAGGTAGAC GCTCGGAGGT TCGCAGCTTT CTGCTCCCCG  600
AACCTGCTGC CTCTGGCCAC AGTGGGTGCT GACATCACAA TCAACAGGGA GCTGGTGCGG  660
AAGGTGGACG GGAAGGCTGG GCTGGTGGTG CACAGCAGCA TGGAGCAGGA CGTGGGCCTG  720
CTGCGCCTCT ACCCTGGGAT CCCTGCCGCC CTGGTTCGGG CCTTCTTGCA GCCTCCCCTG  780
AAGGGCGTGG TCATGGAGAC CTTCGGTTCA GGGAACGGAC CCACCAAGCC CGACCTGCTG  840
CAGGAGCTGC GGGTGGCCAC CGAGCGCGGC CTGGTCATCG TCAACTGTAC CCAGTGCCTC  900
CAGGGGGCTG TGACCACAGA CTATGCAGCT GGCATGGCCA TGGCGGGAGC CGGCGTCATC  960
TCAGGCTTCG ACATGACATC GGAGGCCGCC CTGGCCAAGC TATCGTATGT GCTGGGCCAG 1020
CCAGGGCTGA GCCTGGATGT CAGGAAGGAG CTGCTGACCA AGGACCTTCG GGGGGAGATG 1080
ACGCCACCCT CGGTG                                                   1095
```

(16) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH:1928 base pairs
  - (B) TYPE:nucleic acid
  - (C) STRANDEDNESS:double
  - (D) TOPOLOGY:linear
- (ii) MOLECULE TYPE:cDNA to mRNA
- (iii) HYPOTHETICAL:No
- (iv) ANTI-SENSE:No
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM:guinea pig
  - (F) TISSUE TYPE:liver
- (iX) FEATURE:
  - (A) NAME/KEY:5'UTR
  - (B) LOCATION:1..19
  - (C) IDENTIFICATION METHODS:S
  - (A) NAME/KEY:mat peptide
  - (B) LOCATION:20..1714
  - (C) IDENTIFICATION METHODS:S
  - (A) NAME/KEY:3'UTR
  - (B) LOCATION:1715..1928
  - (C) IDENTIFICATION METHODS:S
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
GAGTGGCTTA GCCGCAGGC ATG GCG CGC GCA TCA GGC TCC GAG AGG CAC          49
                     Met Ala Arg Ala Ser Gly Ser Glu Arg His
                     1               5                  10
CTG CTG CTC ATC TAC ACT GGC GGC ACT TTG GGC ATG CAG AGC AAG GGC      97
Leu Leu Leu Ile Tyr Thr Gly Gly Thr Leu Gly Met Gln Ser Lys Gly
                15                  20                  25
GGG GTG CTC GTC CCC GGC CCA GGC CTG GTC ACT CTG CTG CGG ACC CTG     145
Gly Val Leu Val Pro Gly Pro Gly Leu Val Thr Leu Leu Arg Thr Leu
            30                  35                  40
CCC ATG TTC CAT GAC AAG GAG TTC GCC CAG GCC CAG GGC CTC CCT GAC     193
Pro Met Phe His Asp Lys Glu Phe Ala Gln Ala Gln Gly Leu Pro Asp
        45                  50                  55
CAT GCT CTG GCG CTG CCC CCT GCC AGC CAC GGC CCC AGG GTC CTC TAC     241
```

```
His Ala Leu Ala Leu Pro Pro Ala Ser His Gly Pro Arg Val Leu Tyr
 60                  65                  70
ACG GTG CTG GAG TGC CAG CCC CTC TTG GAT TCC AGC GAC ATG ACC ATC    289
Thr Val Leu Glu Cys Gln Pro Leu Leu Asp Ser Ser Asp Met Thr Ile
75                  80                  85                  90
GAT GAT TGG ATT CGC ATA GCC AAG ATC ATA GAG AGG CAC TAT GAG CAG    337
Asp Asp Trp Ile Arg Ile Ala Lys Ile Ile Glu Arg His Tyr Glu Gln
                95                 100                 105
TAC CAA GGC TTT GTG GTT ATC CAC GGC ACC GAC ACC ATG GCC TTT GGG    385
Tyr Gln Gly Phe Val Val Ile His Gly Thr Asp Thr Met Ala Phe Gly
            110                 115                 120
GCC TCC ATG CTG TCC TTC ATG CTG GAA AAC CTG CAC AAA CCA GTC ATC    433
Ala Ser Met Leu Ser Phe Met Leu Glu Asn Leu His Lys Pro Val Ile
        125                 130                 135
CTC ACT GGC GCC CAG GTG CCA ATC CGT GTG CTG TGG AAT GAC GCC CGG    481
Leu Thr Gly Ala Gln Val Pro Ile Arg Val Leu Trp Asn Asp Ala Arg
    140                 145                 150
GAA AAC CTG CTG GGG GCG TTG CTT GTG GCC GGC CAA TAC ATC ATC CCT    529
Glu Asn Leu Leu Gly Ala Leu Leu Val Ala Gly Gln Tyr Ile Ile Pro
155                 160                 165                 170
GAG GTC TGC CTG TTT ATG AAC AGT CAG CTG TTT CGG GGA AAC CGG GTA    577
Glu Val Cys Leu Phe Met Asn Ser Gln Leu Phe Arg Gly Asn Arg Val
                175                 180                 185
ACC AAG GTG GAC TCC CAG AAG TTT GAG GCC TTC TGC TCC CCC AAT CTG    625
Thr Lys Val Asp Ser Gln Lys Phe Glu Ala Phe Cys Ser Pro Asn Leu
            190                 195                 200
TCC CCA CTA GCC ACT GTG GGC GCG GAT GTC ACA ATT GCC TGG GAC CTG    673
Ser Pro Leu Ala Thr Val Gly Ala Asp Val Thr Ile Ala Trp Asp Leu
        205                 210                 215
GTG CGC AAG GTC AAC TGG AAG GAC CCG CTG GTG GTG CAC AGC AAC ATG    721
Val Arg Lys Val Asn Trp Lys Asp Pro Leu Val Val His Ser Asn Met
    220                 225                 230
GAG CAC GAC GTG GCA CTG CTG CGC CTC TAC CCT GGC ATC CCG GCC TCC    769
Glu His Asp Val Ala Leu Leu Arg Leu Tyr Pro Gly Ile Pro Ala Ser
235                 240                 245                 250
CTG GTC CGG GCA TTC CTG CAG CCC CCG CTC AAG GGC GTG GTC CTG GAG    817
Leu Val Arg Ala Phe Leu Gln Pro Pro Leu Lys Gly Val Val Leu Glu
                255                 260                 265
ACC TTC GGC TCT GGC AAC GGG CCG AGC AAG CCC GAC CTG CTG CAG GAG    865
Thr Phe Gly Ser Gly Asn Gly Pro Ser Lys Pro Asp Leu Leu Gln Glu
            270                 275                 280
TTG CGG GCC GCG GCC CAG CGC GGC CTC ATC ATG GTC AAC TGC AGC CAG    913
Leu Arg Ala Ala Ala Gln Arg Gly Leu Ile Met Val Asn Cys Ser Gln
        285                 290                 295
TGC CTG CGG GGG TCT GTG ACC CCG GGC TAT GCC ACG AGC TTG GCG GGC    961
Cys Leu Arg Gly Ser Val Thr Pro Gly Tyr Ala Thr Ser Leu Ala Gly
    300                 305                 310
GCC AAC ATC GTG TCC GGC TTA GAC ATG ACC TCA GAG GCC GCG CTG GCT   1009
Ala Asn Ile Val Ser Gly Leu Asp Met Thr Ser Glu Ala Ala Leu Ala
315                 320                 325                 330
AAG CTG TCC TAC GTG TTG GGC CTG CCG GAG CTG AGC CTG GAG CGC AGG   1057
Lys Leu Ser Tyr Val Leu Gly Leu Pro Glu Leu Ser Leu Glu Arg Arg
                335                 340                 345
CAG GAG CTG CTG GCC AAG GAT CTT CGC GGG GAA ATG ACA CTG CCC ACG   1105
Gln Glu Leu Leu Ala Lys Asp Leu Arg Gly Glu Met Thr Leu Pro Thr
            350                 355                 360
GCA GAC CTG CAC CAG TCC TCT CCG CCG GGC AGC ACA CTG GGG CAA GGT   1153
```

```
Ala Asp Leu His Gln Ser Ser Pro Pro Gly Ser Thr Leu Gly Gln Gly
          365                 370                 375
GTC GCC CGG CTC TTT AGT CTG TTC GGT TGC CAG GAG GAA GAT TCG GTG   1201
Val Ala Arg Leu Phe Ser Leu Phe Gly Cys Gln Glu Glu Asp Ser Val
    380                 385                 390
CAG GAC GCC GTG ATG CCC AGC CTG GCC CTG GCC TTG GCC CAT GCT GGT   1249
Gln Asp Ala Val Met Pro Ser Leu Ala Leu Ala Leu Ala His Ala Gly
395                 400                 405                 410
GAA CTC GAG GCT CTG CAG GCA CTT ATG GAG CTG GGC AGT GAC CTG CGC   1297
Glu Leu Glu Ala Leu Gln Ala Leu Met Glu Leu Gly Ser Asp Leu Arg
                415                 420                 425
CTA AAG GAC TCT AAT GGC CAA ACC CTG TTG CAT GTG GCT GCT CGG AAT   1345
Leu Lys Asp Ser Asn Gly Gln Thr Leu Leu His Val Ala Ala Arg Asn
            430                 435                 440
GGG CGT GAT GGC GTG GTC ACC ATG CTG CTG CAC AGA GGC ATG GAT GTC   1393
Gly Arg Asp Gly Val Val Thr Met Leu Leu His Arg Gly Met Asp Val
        445                 450                 455
AAT GCC CGA GAC CGA GAC GGC CTC AGC CCA CTG CTG TTG GCT GTA CAG   1441
Asn Ala Arg Asp Arg Asp Gly Leu Ser Pro Leu Leu Leu Ala Val Gln
    460                 465                 470
GGC AGG CAT CGG GAA TGC ATC AGG CTG CTG CGG AAG GCT GGG GCC TGC   1489
Gly Arg His Arg Glu Cys Ile Arg Leu Leu Arg Lys Ala Gly Ala Cys
475                 480                 485                 490
CTG TCC CCC CAG GAC CTG AAG GAT GCA GGG ACC GAG CTG TGC AGG CTG   1537
Leu Ser Pro Gln Asp Leu Lys Asp Ala Gly Thr Glu Leu Cys Arg Leu
                495                 500                 505
GCA TCC AGG GCT GAC ATG GAA GGC CTG CAG GCA TGG GGG CAG GCT GGG   1585
Ala Ser Arg Ala Asp Met Glu Gly Leu Gln Ala Trp Gly Gln Ala Gly
            510                 515                 520
GCC GAC CTG CAG CAG CCG GGC TAT GAT GGG CGC AGC GCT CTG TGT GTC   1633
Ala Asp Leu Gln Gln Pro Gly Tyr Asp Gly Arg Ser Ala Leu Cys Val
        525                 530                 535
GCA GAA GCA GCC GGG AAC CAG GAG GTG CTG GCC CTT CTG CGG AAC CTG   1681
Ala Glu Ala Ala Gly Asn Gln Glu Val Leu Ala Leu Leu Arg Asn Leu
    540                 545                 550
GCA CTT GTA GGC CCG GAA GTG CCG CCT GCC ATC TGATCGCCAG CAATCCCGCT 1734
Ala Leu Val Gly Pro Glu Val Pro Pro Ala Ile
555                 560                 565
GTGGTGTGAG CCACTCCGCC ATCTGCTGCT TTGACCCACT CGAGGGACCC TAGCACACGA 1794
CCCCCCAGCA GGATGCACCC CACTACTTAG AGTATACCCC AGGCTGGCTC AGTGACAAGC 1854
TGCAAAGGTC TTTGTTGGCA GAACAGCAAT AAAGTAACTA CAGAGTGGCC AAAAAAAAAA 1914
AAAAAAAAAA AAAA                                                  1928
```

(17) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2096 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA to mRNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: human
  - (F) TISSUE TYPE: liver

(ix) FEATURE:
(A) NAME/KEY: 5'UTR
(B) LOCATION: 1..92
(C) IDENTIFICATION METHODS: S
(A) NAME/KEY: mat peptide
(B) LOCATION: 93..1811
(C) IDENTIFICATION METHODS: S
(A) NAME/KEY: 3'UTR
(B) LOCATION: 1812..2096
(C) IDENTIFICATION METHODS: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
CGCCCCGGGC CTCCTCCGCG CAGTCCCTGA GTCCCGCAGG CCCTGCGTCC CCGCTGCACA   60
CCCCCGTCCA CTCCCGTGGT CCCCGGTCCG GC ATG GCG CGC GCG GTG GGG CCC     113
                                    Met Ala Arg Ala Val Gly Pro
                                    1               5
GAG CGG AGG CTG CTG GCC GTC TAC ACC GGC GGC ACC ATT GGC ATG CGG     161
Glu Arg Arg Leu Leu Ala Val Tyr Thr Gly Gly Thr Ile Gly Met Arg
        10                  15                  20
AGT GAG CTC GGC GTG CTT GTG CCC GGG ACG GGC CTG GCT GCC ATC CTG     209
Ser Glu Leu Gly Val Leu Val Pro Gly Thr Gly Leu Ala Ala Ile Leu
    25                  30                  35
AGG ACA CTG CCC ATG TTC CAT GAC GAG GAG CAC GCC CGA GCC CGC GGC     257
Arg Thr Leu Pro Met Phe His Asp Glu Glu His Ala Arg Ala Arg Gly
40                  45                  50                  55
CTC TCT GAG GAC ACC CTG GTG CTA CCC CCG GAC AGC CGC AAC CAG AGG     305
Leu Ser Glu Asp Thr Leu Val Leu Pro Pro Asp Ser Arg Asn Gln Arg
                60                  65                  70
ATC CTC TAC ACC GTG CTG GAG TGC CAG CCC CTC TTC GAC TCC AGT GAC     353
Ile Leu Tyr Thr Val Leu Glu Cys Gln Pro Leu Phe Asp Ser Ser Asp
            75                  80                  85
ATG ACC ATC GCT GAG TGG GTT CGC GTT GCC CAG ACC ATC AAG AGG CAC     401
Met Thr Ile Ala Glu Trp Val Arg Val Ala Gln Thr Ile Lys Arg His
        90                  95                  100
TAC GAG CAG TAC CAC GGC TTT GTG GTC ATC CAC GGC ACC GAC ACC ATG     449
Tyr Glu Gln Tyr His Gly Phe Val Val Ile His Gly Thr Asp Thr Met
    105                 110                 115
GCC TTT GCT GCC TCG ATG CTG TCC TTC ATG CTG GAG AAC CTG CAG AAG     497
Ala Phe Ala Ala Ser Met Leu Ser Phe Met Leu Glu Asn Leu Gln Lys
120                 125                 130                 135
ACT GTC ATC CTC ACT GGG GCC CAG GTG CCC ATC CAT GCC CTG TGG AGC     545
Thr Val Ile Leu Thr Gly Ala Gln Val Pro Ile His Ala Leu Trp Ser
                140                 145                 150
GAC GGC CGT GAG AAC CTG CTG GGG GCA CTG CTC ATG GCT GGC CAG TAT     593
Asp Gly Arg Glu Asn Leu Leu Gly Ala Leu Leu Met Ala Gly Gln Tyr
            155                 160                 165
GTG ATC CCA GAG GTC TGC CTT TTC TTC CAG AAT CAG CTG TTT CGG GGC     641
Val Ile Pro Glu Val Cys Leu Phe Phe Gln Asn Gln Leu Phe Arg Gly
        170                 175                 180
AAC CGG GCA ACC AAG GTA GAC GCT CGG AGG TTC GCA GCT TTC TGC TCC     689
Asn Arg Ala Thr Lys Val Asp Ala Arg Arg Phe Ala Ala Phe Cys Ser
    185                 190                 195
CCG AAC CTG CTG CCT CTG GCC ACA GTG GGT GCT GAC ATC ACA ATC AAC     737
Pro Asn Leu Leu Pro Leu Ala Thr Val Gly Ala Asp Ile Thr Ile Asn
200                 205                 210                 215
AGG GAG CTG GTG CGG AAG GTG GAC GGG AAG GCT GGG CTG GTG GTG CAC     785
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Arg Glu Leu Val Arg Lys Val Asp Gly Lys Ala Gly Leu Val Val His
                220                 225                 230
AGC AGC ATG GAG CAG GAC GTG GGC CTG CTG CGC CTC TAC CCT GGG ATC  833
Ser Ser Met Glu Gln Asp Val Gly Leu Leu Arg Leu Tyr Pro Gly Ile
            235                 240                 245
CCT GCC GCC CTG GTT CGG GCC TTC TTG CAG CCT CCC CTG AAG GGC GTG  881
Pro Ala Ala Leu Val Arg Ala Phe Leu Gln Pro Pro Leu Lys Gly Val
        250                 255                 260
GTC ATG GAG ACC TTC GGT TCA GGG AAC GGA CCC ACC AAG CCC GAC CTG  929
Val Met Glu Thr Phe Gly Ser Gly Asn Gly Pro Thr Lys Pro Asp Leu
    265                 270                 275
CTG CAG GAG CTG CGG GTG GCC ACC GAG CGC GGC CTG GTC ATC GTC AAC  977
Leu Gln Glu Leu Arg Val Ala Thr Glu Arg Gly Leu Val Ile Val Asn
280                 285                 290                 295
TGT ACC CAC TGC CTC CAG GGG GCT GTG ACC ACA GAC TAT GCA GCT GGC 1025
Cys Thr His Cys Leu Gln Gly Ala Val Thr Thr Asp Tyr Ala Ala Gly
                300                 305                 310
ATG GCC ATG GCG GGA GCC GGC GTC ATC TCA GGC TTC GAC ATG ACA TCG 1073
Met Ala Met Ala Gly Ala Gly Val Ile Ser Gly Phe Asp Met Thr Ser
            315                 320                 325
GAG GCC GCC CTG GCC AAG CTA TCG TAT GTG CTG GGC CAG CCA GGG CTG 1121
Glu Ala Ala Leu Ala Lys Leu Ser Tyr Val Leu Gly Gln Pro Gly Leu
        330                 335                 340
AGC CTG GAT GTC AGG AAG GAG CTG CTG ACC AAG GAC CTT CGG GGG GAG 1169
Ser Leu Asp Val Arg Lys Glu Leu Leu Thr Lys Asp Leu Arg Gly Glu
    345                 350                 355
ATG ACG CCA CCC TCG GTG GAA GAG CGC CGG CCC TCA CTG CAG GGC AAC 1217
Met Thr Pro Pro Ser Val Glu Glu Arg Arg Pro Ser Leu Gln Gly Asn
360                 365                 370                 375
ACG CTG GGC GGT GGG GTC TCC TGG CTC CTC AGT CTG AGC GGC AGC CAG 1265
Thr Leu Gly Gly Gly Val Ser Trp Leu Leu Ser Leu Ser Gly Ser Gln
                380                 385                 390
GAG GCA GAT GCC CTG CGG AAT GCC CTG GTG CCC AGC CTG GCC TGT GCT 1313
Glu Ala Asp Ala Leu Arg Asn Ala Leu Val Pro Ser Leu Ala Cys Ala
            395                 400                 405
GCT GCC CAC GCC GGT GAC GTG GAG GCG CTG CAG GCG CTT GTG GAG CTG 1361
Ala Ala His Ala Gly Asp Val Glu Ala Leu Gln Ala Leu Val Glu Leu
        410                 415                 420
GGC AGT GAC CTG GGC CTG GTG GAC TTT AAC GGC CAA ACC CCA CTG CAC 1409
Gly Ser Asp Leu Gly Leu Val Asp Phe Asn Gly Gln Thr Pro Leu His
    425                 430                 435
GCG GCC GCC CGG GGA GGC CAC ACA GAG GCA GTC ACC ATG CTG CTG CAG 1457
Ala Ala Ala Arg Gly Gly His Thr Glu Ala Val Thr Met Leu Leu Gln
440                 445                 450                 455
AGA GGT GTG GAC GTG AAC ACC CGG GAC ACG GAT GGC TTC AGC CCG CTG 1505
Arg Gly Val Asp Val Asn Thr Arg Asp Thr Asp Gly Phe Ser Pro Leu
                460                 465                 470
CTG CTG GCC GTG CGG GGC AGG CAT CCG GGT GTC ATT GGG TTG CTG CGG 1553
Leu Leu Ala Val Arg Gly Arg His Pro Gly Val Ile Gly Leu Leu Arg
            475                 480                 485
GAA GCC GGG GCC TCC CTG TCC ACC CAG GAG CTG GAG GAA GCA GGG ACG 1601
Glu Ala Gly Ala Ser Leu Ser Thr Gln Glu Leu Glu Glu Ala Gly Thr
        490                 495                 500
GAG CTG TGC AGG CTG GCA TAC AGG GCC GAC CTC GAA GGC CTG CAG GTG 1649
Glu Leu Cys Arg Leu Ala Tyr Arg Ala Asp Leu Glu Gly Leu Gln Val
    505                 510                 515
TGG TGG CAG GCA GGG GCT GAC CTG GGG CAG CCG GGC TAT GAC GGG CAC 1697
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Trp Trp Gln Ala Gly Ala Asp Leu Gly Gln Pro Gly Tyr Asp Gly His
520                 525                 530                 535
AGC GCC CTG CAC GTC GCA GAG GCA GCC GGG AAC CTG GCA GTG GTG GCC   1745
Ser Ala Leu His Val Ala Glu Ala Ala Gly Asn Leu Ala Val Val Ala
                540                 545                 550
TTT CTA CAG AGC CTG GAG GGT GCG GTT GGT GCC CAG GCC CCA TGC CCA   1793
Phe Leu Gln Ser Leu Glu Gly Ala Val Gly Ala Gln Ala Pro Cys Pro
            555                 560                 565
GAA GTG CTG CCT GGT GTC TAACCTGAAG GCGTCCTGCT GCAGTATAAG          1841
Glu Val Leu Pro Gly Val
        570
CCATTCCTTC CTCCCATGAC CTGCTGGAGG GGTCTCAGGC ATGACCCCAC TGCTGGGGCT 1901
GCTTCCCAGC CTGCTCTCAT GTAAAGCCTG AAGGCCTTTG TTGGGCAGGA CGGCAATAAA 1961
GTCTCTGACA TCCCCTCACC AGGTCTGTAC AGCCTGGCTC TGAGAGGCTC TGTCTGGGTC 2021
CGGGACTGTG AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 2081
AAAAAAAAAA AAAAA                                                  2096
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(18) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH:1695 base pairs
  - (B) TYPE:nucleic acid
  - (C) STRANDEDNESS:double
  - (D) TOPOLOGY:linear
- (ii) MOLECULE TYPE:cDNA to mRNA
- (iii) HYPOTHETICAL:No
- (iv) ANTI-SENSE:No
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM:guinea pig
  - (F) TISSUE TYPE:liver
- (iX) FEATURE:
  - (A) NAME/KEY:mat peptide
  - (B) LOCATIOM:1..1695
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

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ATG GCG CGC GCA TCA GGC TCC GAG AGG CAC CTG CTG CTC ATC TAC ACT     48
Met Ala Arg Ala Ser Gly Ser Glu Arg His Leu Leu Leu Ile Tyr Thr
1               5                   10                  15
GGC GGC ACT TTG GGC ATG CAG AGC AAG GGC GGG GTG CTC GTC CCC GGC     96
Gly Gly Thr Leu Gly Met Gln Ser Lys Gly Gly Val Leu Val Pro Gly
            20                  25                  30
CCA GGC CTG GTC ACT CTG CTG CGG ACC CTG CCC ATG TTC CAT GAC AAG    144
Pro Gly Leu Val Thr Leu Leu Arg Thr Leu Pro Met Phe His Asp Lys
        35                  40                  45
GAG TTC GCC CAG GCC CAG GGC CTC CCT GAC CAT GCT CTG GCG CTG CCC    192
Glu Phe Ala Gln Ala Gln Gly Leu Pro Asp His Ala Leu Ala Leu Pro
    50                  55                  60
CCT GCC AGC CAC GGC CCC AGG GTC CTC TAC ACG GTG CTG GAG TGC CAG    240
Pro Ala Ser His Gly Pro Arg Val Leu Tyr Thr Val Leu Glu Cys Gln
65                  70                  75                  80
CCC CTC TTG GAT TCC AGC GAC ATG ACC ATC GAT GAT TGG ATT CGC ATA    288
Pro Leu Leu Asp Ser Ser Asp Met Thr Ile Asp Asp Trp Ile Arg Ile
                85                  90                  95
GCC AAG ATC ATA GAG AGG CAC TAT GAG CAG TAC CAA GGC TTT GTG GTT    336
Ala Lys Ile Ile Glu Arg His Tyr Glu Gln Tyr Gln Gly Phe Val Val
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                   100                 105                 110
ATC CAC GGC ACC GAC ACC ATG GCC TTT GGG GCC TCC ATG CTG TCC TTC     384
Ile His Gly Thr Asp Thr Met Ala Phe Gly Ala Ser Met Leu Ser Phe
           115                 120                 125
ATG CTG GAA AAC CTG CAC AAA CCA GTC ATC CTC ACT GGC GCC CAG GTG     432
Met Leu Glu Asn Leu His Lys Pro Val Ile Leu Thr Gly Ala Gln Val
       130                 135                 140
CCA ATC CGT GTG CTG TGG AAT GAC GCC CGG GAA AAC CTG CTG GGG GCG     480
Pro Ile Arg Val Leu Trp Asn Asp Ala Arg Glu Asn Leu Leu Gly Ala
145                 150                 155                 160
TTG CTT GTG GCC GGC CAA TAC ATC ATC CCT GAG GTC TGC CTG TTT ATG     528
Leu Leu Val Ala Gly Gln Tyr Ile Ile Pro Glu Val Cys Leu Phe Met
                   165                 170                 175
AAC AGT CAG CTG TTT CGG GGA AAC CGG GTA ACC AAG GTG GAC TCC CAG     576
Asn Ser Gln Leu Phe Arg Gly Asn Arg Val Thr Lys Val Asp Ser Gln
           180                 185                 190
AAG TTT GAG GCC TTC TGC TCC CCC AAT CTG TCC CCA CTA GCC ACT GTG     624
Lys Phe Glu Ala Phe Cys Ser Pro Asn Leu Ser Pro Leu Ala Thr Val
       195                 200                 205
GGC GCG GAT GTC ACA ATT GCC TGG GAC CTG GTG CGC AAG GTC AAC TGG     672
Gly Ala Asp Val Thr Ile Ala Trp Asp Leu Val Arg Lys Val Asn Trp
   210                 215                 220
AAG GAC CCG CTG GTG GTG CAC AGC AAC ATG GAG CAC GAC GTG GCA CTG     720
Lys Asp Pro Leu Val Val His Ser Asn Met Glu His Asp Val Ala Leu
225                 230                 235                 240
CTG CGC CTC TAC CCT GGC ATC CCG GCC TCC CTG GTC CGG GCA TTC CTG     768
Leu Arg Leu Tyr Pro Gly Ile Pro Ala Ser Leu Val Arg Ala Phe Leu
                   245                 250                 255
CAG CCC CCG CTC AAG GGC GTG GTC CTG GAG ACC TTC GGC TCT GGC AAC     816
Gln Pro Pro Leu Lys Gly Val Val Leu Glu Thr Phe Gly Ser Gly Asn
           260                 265                 270
GGG CCG AGC AAG CCC GAC CTG CTG CAG GAG TTG CGG GCC GCG GCC CAG     864
Gly Pro Ser Lys Pro Asp Leu Leu Gln Glu Leu Arg Ala Ala Ala Gln
       275                 280                 285
CGC GGC CTC ATC ATG GTC AAC TGC AGC CAG TGC CTG CGG GGG TCT GTG     912
Arg Gly Leu Ile Met Val Asn Cys Ser Gln Cys Leu Arg Gly Ser Val
   290                 295                 300
ACC CCG GGC TAT GCC ACG AGC TTG GCG GGC GCC AAC ATC GTG TCC GGC     960
Thr Pro Gly Tyr Ala Thr Ser Leu Ala Gly Ala Asn Ile Val Ser Gly
305                 310                 315                 320
TTA GAC ATG ACC TCA GAG GCC GCG CTG GCT AAG CTG TCC TAC GTG TTG    1008
Leu Asp Met Thr Ser Glu Ala Ala Leu Ala Lys Leu Ser Tyr Val Leu
                   325                 330                 335
GGC CTG CCG GAG CTG AGC CTG GAG CGC AGG CAG GAG CTG CTG GCC AAG    1056
Gly Leu Pro Glu Leu Ser Leu Glu Arg Arg Gln Glu Leu Leu Ala Lys
           340                 345                 350
GAT CTT CGC GGG GAA ATG ACA CTG CCC ACG GCA GAC CTG CAC CAG TCC    1104
Asp Leu Arg Gly Glu Met Thr Leu Pro Thr Ala Asp Leu His Gln Ser
       355                 360                 365
TCT CCG CCG GGC AGC ACA CTG GGG CAA GGT GTC GCC CGG CTC TTT AGT    1152
Ser Pro Pro Gly Ser Thr Leu Gly Gln Gly Val Ala Arg Leu Phe Ser
   370                 375                 380
CTG TTC GGT TGC CAG GAG GAA GAT TCG GTG CAG GAC GCC GTG ATG CCC    1200
Leu Phe Gly Cys Gln Glu Glu Asp Ser Val Gln Asp Ala Val Met Pro
385                 390                 395                 400
AGC CTG GCC CTG GCC TTG GCC CAT GCT GGT GAA CTC GAG GCT CTG CAG    1248
Ser Leu Ala Leu Ala Leu Ala His Ala Gly Glu Leu Glu Ala Leu Gln
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                    405                 410                 415
GCA CTT ATG GAG CTG GGC AGT GAC CTG CGC CTA AAG GAC TCT AAT GGC   1296
Ala Leu Met Glu Leu Gly Ser Asp Leu Arg Leu Lys Asp Ser Asn Gly
                420                 425                 430
CAA ACC CTG TTG CAT GTG GCT GCT CGG AAT GGG CGT GAT GGC GTG GTC   1344
Gln Thr Leu Leu His Val Ala Ala Arg Asn Gly Arg Asp Gly Val Val
            435                 440                 445
ACC ATG CTG CTG CAC AGA GGC ATG GAT GTC AAT GCC CGA GAC CGA GAC   1392
Thr Met Leu Leu His Arg Gly Met Asp Val Asn Ala Arg Asp Arg Asp
        450                 455                 460
GGC CTC AGC CCA CTG CTG TTG GCT GTA CAG GGC AGG CAT CGG GAA TGC   1440
Gly Leu Ser Pro Leu Leu Leu Ala Val Gln Gly Arg His Arg Glu Cys
465                 470                 475                 480
ATC AGG CTG CTG CGG AAG GCT GGG GCC TGC CTG TCC CCC CAG GAC CTG   1488
Ile Arg Leu Leu Arg Lys Ala Gly Ala Cys Leu Ser Pro Gln Asp Leu
                485                 490                 495
AAG GAT GCA GGG ACC GAG CTG TGC AGG CTG GCA TCC AGG GCT GAC ATG   1536
Lys Asp Ala Gly Thr Glu Leu Cys Arg Leu Ala Ser Arg Ala Asp Met
            500                 505                 510
GAA GGC CTG CAG GCA TGG GGG CAG GCT GGG GCC GAC CTG CAG CAG CCG   1584
Glu Gly Leu Gln Ala Trp Gly Gln Ala Gly Ala Asp Leu Gln Gln Pro
        515                 520                 525
GGC TAT GAT GGG CGC AGC GCT CTG TGT GTC GCA GAA GCA GCC GGG AAC   1632
Gly Tyr Asp Gly Arg Ser Ala Leu Cys Val Ala Glu Ala Ala Gly Asn
    530                 535                 540
CAG GAG GTG CTG GCC CTT CTG CGG AAC CTG GCA CTT GTA GGC CCG GAA   1680
Gln Glu Val Leu Ala Leu Leu Arg Asn Leu Ala Leu Val Gly Pro Glu
545                 550                 555                 560
GTG CCG CCT GCC ATC                                             1695
Val Pro Pro Ala Ile
                565
```

(19) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH:1719 base pairs
  - (B) TYPE:nucleic acid
  - (C) STRANDEDNESS:double
  - (D) TOPOLOGY:linear
- (ii) MOLECULE TYPE:cDNA to mRNA
- (iii) HYPOTHETICAL:No
- (iv) ANTI-SENSE:No
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM:human
  - (F) TISSUE TYPE:liver
- (iX) FEATURE:
  - (A) NAME/KEY:mat peptide
  - (B) LOCATION:1..1719
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

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ATG GCG CGC GCG GTG GGG CCC GAG CGG AGG CTG CTG GCC GTC TAC ACC     48
Met Ala Arg Ala Val Gly Pro Glu Arg Arg Leu Leu Ala Val Tyr Thr
1               5                   10                  15
GGC GGC ACC ATT GGC ATG CGG AGT GAG CTC GGC GTG CTT GTG CCC GGG     96
Gly Gly Thr Ile Gly Met Arg Ser Glu Leu Gly Val Leu Val Pro Gly
            20                  25                  30
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ACG GGC CTG GCT GCC ATC CTG AGG ACA CTG CCC ATG TTC CAT GAC GAG     144
Thr Gly Leu Ala Ala Ile Leu Arg Thr Leu Pro Met Phe His Asp Glu
          35                  40                  45
GAG CAC GCC CGA GCC CGC GGC CTC TCT GAG GAC ACC CTG GTG CTA CCC     192
Glu His Ala Arg Ala Arg Gly Leu Ser Glu Asp Thr Leu Val Leu Pro
      50                  55                  60
CCG GAC AGC CGC AAC CAG AGG ATC CTC TAC ACC GTG CTG GAG TGC CAG     240
Pro Asp Ser Arg Asn Gln Arg Ile Leu Tyr Thr Val Leu Glu Cys Gln
65                  70                  75                  80
CCC CTC TTC GAC TCC AGT GAC ATG ACC ATC GCT GAG TGG GTT CGC GTT     288
Pro Leu Phe Asp Ser Ser Asp Met Thr Ile Ala Glu Trp Val Arg Val
                  85                  90                  95
GCC CAG ACC ATC AAG AGG CAC TAC GAG CAG TAC CAC GGC TTT GTG GTC     336
Ala Gln Thr Ile Lys Arg His Tyr Glu Gln Tyr His Gly Phe Val Val
             100                 105                 110
ATC CAC GGC ACC GAC ACC ATG GCC TTT GCT GCC TCG ATG CTG TCC TTC     384
Ile His Gly Thr Asp Thr Met Ala Phe Ala Ala Ser Met Leu Ser Phe
         115                 120                 125
ATG CTG GAG AAC CTG CAG AAG ACT GTC ATC CTC ACT GGG GCC CAG GTG     432
Met Leu Glu Asn Leu Gln Lys Thr Val Ile Leu Thr Gly Ala Gln Val
     130                 135                 140
CCC ATC CAT GCC CTG TGG AGC GAC GGC CGT GAG AAC CTG CTG GGG GCA     480
Pro Ile His Ala Leu Trp Ser Asp Gly Arg Glu Asn Leu Leu Gly Ala
145                 150                 155                 160
CTG CTC ATG GCT GGC CAG TAT GTG ATC CCA GAG GTC TGC CTT TTC TTC     528
Leu Leu Met Ala Gly Gln Tyr Val Ile Pro Glu Val Cys Leu Phe Phe
                 165                 170                 175
CAG AAT CAG CTG TTT CGG GGC AAC CGG GCA ACC AAG GTA GAC GCT CGG     576
Gln Asn Gln Leu Phe Arg Gly Asn Arg Ala Thr Lys Val Asp Ala Arg
             180                 185                 190
AGG TTC GCA GCT TTC TGC TCC CCG AAC CTG CTG CCT CTG GCC ACA GTG     624
Arg Phe Ala Ala Phe Cys Ser Pro Asn Leu Leu Pro Leu Ala Thr Val
         195                 200                 205
GGT GCT GAC ATC ACA ATC AAC AGG GAG CTG GTG CGG AAG GTG GAC GGG     672
Gly Ala Asp Ile Thr Ile Asn Arg Glu Leu Val Arg Lys Val Asp Gly
     210                 215                 220
AAG GCT GGG CTG GTG GTG CAC AGC AGC ATG GAG CAG GAC GTG GGC CTG     720
Lys Ala Gly Leu Val Val His Ser Ser Met Glu Gln Asp Val Gly Leu
225                 230                 235                 240
CTG CGC CTC TAC CCT GGG ATC CCT GCC GCC CTG GTT CGG GCC TTC TTG     768
Leu Arg Leu Tyr Pro Gly Ile Pro Ala Ala Leu Val Arg Ala Phe Leu
                 245                 250                 255
CAG CCT CCC CTG AAG GGC GTG GTC ATG GAG ACC TTC GGT TCA GGG AAC     816
Gln Pro Pro Leu Lys Gly Val Val Met Glu Thr Phe Gly Ser Gly Asn
             260                 265                 270
GGA CCC ACC AAG CCC GAC CTG CTG CAG GAG CTG CGG GTG GCC ACC GAG     864
Gly Pro Thr Lys Pro Asp Leu Leu Gln Glu Leu Arg Val Ala Thr Glu
         275                 280                 285
CGC GGC CTG GTC ATC GTC AAC TGT ACC CAC TGC CTC CAG GGG GCT GTG     912
Arg Gly Leu Val Ile Val Asn Cys Thr His Cys Leu Gln Gly Ala Val
     290                 295                 300
ACC ACA GAC TAT GCA GCT GGC ATG GCC ATG GCG GGA GCC GGC GTC ATC     960
Thr Thr Asp Tyr Ala Ala Gly Met Ala Met Ala Gly Ala Gly Val Ile
305                 310                 315                 320
TCA GGC TTC GAC ATG ACA TCG GAG GCC GCC CTG GCC AAG CTA TCG TAT    1008
Ser Gly Phe Asp Met Thr Ser Glu Ala Ala Leu Ala Lys Leu Ser Tyr
                 325                 330                 335
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GTG CTG GGC CAG CCA GGG CTG AGC CTG GAT GTC AGG AAG GAG CTG CTG   1056
Val Leu Gly Gln Pro Gly Leu Ser Leu Asp Val Arg Lys Glu Leu Leu
            340                 345                 350
ACC AAG GAC CTT CGG GGG GAG ATG ACG CCA CCC TCG GTG GAA GAG CGC   1104
Thr Lys Asp Leu Arg Gly Glu Met Thr Pro Pro Ser Val Glu Glu Arg
        355                 360                 365
CGG CCC TCA CTG CAG GGC AAC ACG CTG GGC GGT GGG GTC TCC TGG CTC   1152
Arg Pro Ser Leu Gln Gly Asn Thr Leu Gly Gly Gly Val Ser Trp Leu
    370                 375                 380
CTC AGT CTG AGC GGC AGC CAG GAG GCA GAT GCC CTG CGG AAT GCC CTG   1200
Leu Ser Leu Ser Gly Ser Gln Glu Ala Asp Ala Leu Arg Asn Ala Leu
385                 390                 395                 400
GTG CCC AGC CTG GCC TGT GCT GCT GCC CAC GCC GGT GAC GTG GAG GCG   1248
Val Pro Ser Leu Ala Cys Ala Ala Ala His Ala Gly Asp Val Glu Ala
                405                 410                 415
CTG CAG GCG CTT GTG GAG CTG GGC AGT GAC CTG GGC CTG GTG GAC TTT   1296
Leu Gln Ala Leu Val Glu Leu Gly Ser Asp Leu Gly Leu Val Asp Phe
            420                 425                 430
AAC GGC CAA ACC CCA CTG CAC GCG GCC GCC CGG GGA GGC CAC ACA GAG   1344
Asn Gly Gln Thr Pro Leu His Ala Ala Ala Arg Gly Gly His Thr Glu
        435                 440                 445
GCA GTC ACC ATG CTG CTG CAG AGA GGT GTG GAC GTG AAC ACC CGG GAC   1392
Ala Val Thr Met Leu Leu Gln Arg Gly Val Asp Val Asn Thr Arg Asp
    450                 455                 460
ACG GAT GGC TTC AGC CCG CTG CTG CTG GCC GTG CGG GGC AGG CAT CCG   1440
Thr Asp Gly Phe Ser Pro Leu Leu Leu Ala Val Arg Gly Arg His Pro
465                 470                 475                 480
GGT GTC ATT GGG TTG CTG CGG GAA GCC GGG GCC TCC CTG TCC ACC CAG   1488
Gly Val Ile Gly Leu Leu Arg Glu Ala Gly Ala Ser Leu Ser Thr Gln
                485                 490                 495
GAG CTG GAG GAA GCA GGG ACG GAG CTG TGC AGG CTG GCA TAC AGG GCC   1536
Glu Leu Glu Glu Ala Gly Thr Glu Leu Cys Arg Leu Ala Tyr Arg Ala
            500                 505                 510
GAC CTC GAA GGC CTG CAG GTG TGG TGG CAG GCA GGG GCT GAC CTG GGG   1584
Asp Leu Glu Gly Leu Gln Val Trp Trp Gln Ala Gly Ala Asp Leu Gly
        515                 520                 525
CAG CCG GGC TAT GAC GGG CAC AGC GCC CTG CAC GTC GCA GAG GCA GCC   1632
Gln Pro Gly Tyr Asp Gly His Ser Ala Leu His Val Ala Glu Ala Ala
    530                 535                 540
GGG AAC CTG GCA GTG GTG GCC TTT CTA CAG AGC CTG GAG GGT GCG GTT   1680
Gly Asn Leu Ala Val Val Ala Phe Leu Gln Ser Leu Glu Gly Ala Val
545                 550                 555                 560
GGT GCC CAG GCC CCA TGC CCA GAA GTG CTG CCT GGT GTC               1716
Gly Ala Gln Ala Pro Cys Pro Glu Val Leu Pro Gly Val
                565                 570         573
```

Claims

1. A polypeptide which originate from mammal, having L-asparaginase activity.

2. The polypeptide of claim 1, which is obtainable by the expression of a gene originating from mammal.

3. The polypeptide of claim 1, which has amino acid sequences of SEQ ID NOs:1 to 3 (where the symbol "Xaa" means "glutamine" or "threonine");

SEQ ID NO: 1:

Thr Gly Gly Thr
1

SEQ ID NO: 2:

His Gly Thr Asp Thr
1 5

SEQ ID NO: 3:

Gln Cys Leu Xaa Gly.
1 5

4. The polypeptide of claim 1, which has an amino acid sequence selected from the group consisting of SEQ ID NOs: 4 to 9 and homologous amino acid sequences thereunto;

SEQ ID NO: 4:

```
Met Ala Arg Ala Ser Gly Ser Glu Arg His Leu Leu Leu Ile Tyr Thr
1               5                  10                  15
Gly Gly Thr Leu Gly Met Gln Ser Lys Gly Gly Val Leu Val Pro Gly
            20                  25                  30
Pro Gly Leu Val Thr Leu Leu Arg Thr Leu Pro Met Phe His Asp Lys
        35                  40                  45
Glu Phe Ala Gln Ala Gln Gly Leu Pro Asp His Ala Leu Ala Leu Pro
    50                  55                  60
```

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Pro Ala Ser His Gly Pro Arg Val Leu Tyr Thr Val Leu Glu Cys Gln
65                  70                  75                  80
Pro Leu Leu Asp Ser Ser Asp Met Thr Ile Asp Asp Trp Ile Arg Ile
                85                  90                  95
Ala Lys Ile Ile Glu Arg His Tyr Glu Gln Tyr Gln Gly Phe Val Val
            100                 105                 110
Ile His Gly Thr Asp Thr Met Ala Phe Gly Ala Ser Met Leu Ser Phe
        115                 120                 125
Met Leu Glu Asn Leu His Lys Pro Val Ile Leu Thr Gly Ala Gln Val
    130                 135                 140
Pro Ile Arg Val Leu Trp Asn Asp Ala Arg Glu Asn Leu Leu Gly Ala
145                 150                 155                 160
Leu Leu Val Ala Gly Gln Tyr Ile Ile Pro Glu Val Cys Leu Phe Met
                165                 170                 175
Asn Ser Gln Leu Phe Arg Gly Asn Arg Val Thr Lys Val Asp Ser Gln
            180                 185                 190
Lys Phe Glu Ala Phe Cys Ser Pro Asn Leu Ser Pro Leu Ala Thr Val
        195                 200                 205
Gly Ala Asp Val Thr Ile Ala Trp Asp Leu Val Arg Lys Val Asn Trp
    210                 215                 220
Lys Asp Pro Leu Val Val His Ser Asn Met Glu His Asp Val Ala Leu
225                 230                 235                 240
Leu Arg Leu Tyr Pro Gly Ile Pro Ala Ser Leu Val Arg Ala Phe Leu
                245                 250                 255
Gln Pro Pro Leu Lys Gly Val Val Leu Glu Thr Phe Gly Ser Gly Asn
            260                 265                 270
Gly Pro Ser Lys Pro Asp Leu Leu Gln Glu Leu Arg Ala Ala Ala Gln
        275                 280                 285
Arg Gly Leu Ile Met Val Asn Cys Ser Gln Cys Leu Arg Gly Ser Val
    290                 295                 300
Thr Pro Gly Tyr Ala Thr Ser Leu Ala Gly Ala Asn Ile Val Ser Gly
305                 310                 315                 320
Leu Asp Met Thr Ser Glu Ala Ala Leu Ala Lys Leu Ser Tyr Val Leu
                325                 330                 335
Gly Leu Pro Glu Leu Ser Leu Glu Arg Arg Gln Glu Leu Leu Ala Lys
            340                 345                 350
Asp Leu Arg Gly Glu Met Thr Leu Pro Thr Ala
        355                 360         363
```

SEQ ID NO: 5:

```
Met Ala Arg Ala Ser Gly Ser Glu Arg His Leu Leu Leu Ile Tyr Thr
1               5                  10                  15
Gly Gly Thr Leu Gly Met Gln Ser Lys Gly Gly Val Leu Val Pro Gly
            20                  25                  30
Pro Gly Leu Val Thr Leu Leu Arg Thr Leu Pro Met Phe His Asp Lys
        35                  40                  45
Glu Phe Ala Gln Ala Gln Gly Leu Pro Asp His Ala Leu Ala Leu Pro
    50                  55                  60
Pro Ala Ser His Gly Pro Arg Val Leu Tyr Thr Val Leu Glu Cys Gln
65                  70                  75                  80
Pro Leu Leu Asp Ser Ser Asp Met Thr Ile Asp Asp Trp Ile Arg Ile
                85                  90                  95
Ala Lys Ile Ile Glu Arg His Tyr Glu Gln Tyr Gln Gly Phe Val Val
            100                 105                 110
```

```
Ile His Gly Thr Asp Thr Met Ala Phe Gly Ala Ser Met Leu Ser Phe
        115                 120                 125
Met Leu Glu Asn Leu His Lys Pro Val Ile Leu Thr Gly Ala Gln Val
    130                 135                 140
Pro Ile Arg Val Leu Trp Asn Asp Ala Arg Glu Asn Leu Leu Gly Ala
145                 150                 155                 160
Leu Leu Val Ala Gly Gln Tyr Ile Ile Pro Glu Val Cys Leu Phe Met
                165                 170                 175
Asn Ser Gln Leu Phe Arg Gly Asn Arg Val Thr Lys Val Asp Ser Gln
            180                 185                 190
Lys Phe Glu Ala Phe Cys Ser Pro Asn Leu Ser Pro Leu Ala Thr Val
        195                 200                 205
Gly Ala Asp Val Thr Ile Ala Trp Asp Leu Val Arg Lys Val Asn Trp
    210                 215                 220
Lys Asp Pro Leu Val Val His Ser Asn Met Glu His Asp Val Ala Leu
225                 230                 235                 240
Leu Arg Leu Tyr Pro Gly Ile Pro Ala Ser Leu Val Arg Ala Phe Leu
                245                 250                 255
Gln Pro Pro Leu Lys Gly Val Val Leu Glu Thr Phe Gly Ser Gly Asn
            260                 265                 270
Gly Pro Ser Lys Pro Asp Leu Leu Gln Glu Leu Arg Ala Ala Ala Gln
        275                 280                 285
Arg Gly Leu Ile Met Val Asn Cys Ser Gln Cys Leu Arg Gly Ser Val
    290                 295                 300
Thr Pro Gly Tyr Ala Thr Ser Leu Ala Gly Ala Asn Ile Val Ser Gly
305                 310                 315                 320
Leu Asp Met Thr Ser Glu Ala Ala Leu Ala Lys Leu Ser Tyr Val Leu
                325                 330                 335
Gly Leu Pro Glu Leu Ser Leu Glu Arg Arg Gln Glu Leu Leu Ala Lys
            340                 345                 350
Asp Leu Arg Gly Glu Met Thr Leu Pro Thr Ala Asp Leu His Gln Ser
        355                 360                 365
Ser Pro Pro Gly Ser Thr Leu Gly Gln Gly Val Ala Arg Leu Phe Ser
    370                 375                 380
Leu Phe Gly Cys Gln Glu Glu Asp Ser Val Gln Asp Ala Val Met Pro
385                 390                 395                 400
Ser Leu Ala Leu Ala Leu Ala His Ala Gly Glu Leu Glu Ala Leu Gln
                405                 410                 415
Ala Leu Met Glu Leu Gly Ser Asp Leu Arg Leu Lys Asp Ser Asn Gly
            420                 425                 430
Gln Thr Leu Leu His Val Ala Ala Arg Asn Gly Arg Asp Gly Val Val
        435                 440                 445
Thr Met Leu Leu His Arg Gly Met Asp Val Asn Ala Arg Asp Arg Asp
    450                 455                 460
Gly Leu Ser Pro Leu Leu Leu Ala Val Gln Gly Arg His Arg Glu Cys
465                 470                 475                 480
Ile Arg Leu Leu Arg Lys Ala Gly Ala Cys Leu Ser Pro Gln Asp Leu
                485                 490                 495
Lys Asp Ala Gly Thr Glu Leu Cys Arg Leu Ala Ser Arg Ala Asp Met
            500                 505                 510
Glu Gly Leu Gln Ala Trp Gly Gln Ala Gly Ala Asp Leu Gln Gln Pro
        515                 520                 525
Gly Tyr Asp Gly Arg Ser Ala Leu Cys Val Ala Glu Ala Ala Gly Asn
    530                 535                 540
Gln Glu Val Leu Ala Leu Leu Arg Asn Leu Ala Leu Val Gly Pro Glu
545                 550                 555                 560
Val Pro Pro Ala Ile
```

565

SEQ ID NO: 6:

```
Met Ala Arg Ala Val Gly Pro Glu Arg Arg Leu Leu Ala Val Tyr Thr
1               5                  10                  15
Gly Gly Thr Ile Gly Met Arg Ser Glu Leu Gly Val Leu Val Pro Gly
            20                  25                  30
Thr Gly Leu Ala Ala Ile Leu Arg Thr Leu Pro Met Phe His Asp Glu
        35                  40                  45
Glu His Ala Arg Ala Arg Gly Leu Ser Glu Asp Thr Leu Val Leu Pro
    50                  55                  60
Pro Asp Ser Arg Asn Gln Arg Ile Leu Tyr Thr Val Leu Glu Cys Gln
65                  70                  75                  80
Pro Leu Phe Asp Ser Ser Asp Met Thr Ile Ala Glu Trp Val Arg Val
                85                  90                  95
Ala Gln Thr Ile Lys Arg His Tyr Glu Gln Tyr His Gly Phe Val Val
            100                 105                 110
Ile His Gly Thr Asp Thr Met Ala Phe Ala Ala Ser Met Leu Ser Phe
        115                 120                 125
Met Leu Glu Asn Leu Gln Lys Thr Val Ile Leu Thr Gly Ala Gln Val
    130                 135                 140
Pro Ile His Ala Leu Trp Ser Asp Gly Arg Glu Asn Leu Leu Gly Ala
145                 150                 155                 160
Leu Leu Met Ala Gly Gln Tyr Val Ile Pro Glu Val Cys Leu Phe Phe
                165                 170                 175
Gln Asn Gln Leu Phe Arg Gly Asn Arg Ala Thr Lys Val Asp Ala Arg
            180                 185                 190
Arg Phe Ala Ala Phe Cys Ser Pro Asn Leu Leu Pro Leu Ala Thr Val
        195                 200                 205
Gly Ala Asp Ile Thr Ile Asn Arg Glu Leu Val Arg Lys Val Asp Gly
    210                 215                 220
Lys Ala Gly Leu Val Val His Ser Ser Met Glu Gln Asp Val Gly Leu
225                 230                 235                 240
Leu Arg Leu Tyr Pro Gly Ile Pro Ala Ala Leu Val Arg Ala Phe Leu
                245                 250                 255
Gln Pro Pro Leu Lys Gly Val Val Met Glu Thr Phe Gly Ser Gly Asn
            260                 265                 270
Gly Pro Thr Lys Pro Asp Leu Leu Gln Glu Leu Arg Val Ala Thr Glu
        275                 280                 285
Arg Gly Leu Val Ile Val Asn Cys Thr Gln Cys Leu Arg Gly Ala Val
    290                 295                 300
Thr Thr Asp Tyr Ala Ala Gly Met Ala Met Ala Gly Ala Asn Val Ile
305                 310                 315                 320
Ser Gly Phe Asp Met Thr Ser Glu Ala Ala Leu Ala Lys Leu Ser Tyr
                325                 330                 335
Val Leu Gly Gln Pro Gly Leu Ser Leu Asp Val Arg Lys Glu Leu Leu
            340                 345                 350
Thr Lys Asp Leu Arg Gly Glu Met Thr Pro Pro Ser Val
        355                 360                 365
```

SEQ ID NO: 7:

```
Met Ala Arg Ala Val Gly Pro Glu Arg Arg Leu Leu Ala Val Tyr Thr
1               5                  10                  15
Gly Gly Thr Ile Gly Met Arg Ser Glu Leu Gly Val Leu Val Pro Gly
            20                  25                  30
Thr Gly Leu Ala Ala Ile Leu Arg Thr Leu Pro Met Phe His Asp Glu
        35                  40                  45
Glu His Ala Arg Ala Arg Gly Leu Ser Glu Asp Thr Leu Val Leu Pro
    50                  55                  60
Pro Asp Ser Arg Asn Gln Arg Ile Leu Tyr Thr Val Leu Glu Cys Gln
65                  70                  75                  80
Pro Leu Phe Asp Ser Ser Asp Met Thr Ile Ala Glu Trp Val Arg Val
                85                  90                  95
Ala Gln Thr Ile Lys Arg His Tyr Glu Gln Tyr His Gly Phe Val Val
            100                 105                 110
Ile His Gly Thr Asp Thr Met Ala Phe Ala Ala Ser Met Leu Ser Phe
        115                 120                 125
Met Leu Glu Asn Leu Gln Lys Thr Val Ile Leu Thr Gly Ala Gln Val
    130                 135                 140
Pro Ile His Ala Leu Trp Ser Asp Gly Arg Glu Asn Leu Leu Gly Ala
145                 150                 155                 160
Leu Leu Met Ala Gly Gln Tyr Val Ile Pro Glu Val Cys Leu Phe Phe
                165                 170                 175
Gln Asn Gln Leu Phe Arg Gly Asn Arg Ala Thr Lys Val Asp Ala Arg
            180                 185                 190
Arg Phe Ala Ala Phe Cys Ser Pro Asn Leu Leu Pro Leu Ala Thr Val
        195                 200                 205
Gly Ala Asp Ile Thr Ile Asn Arg Glu Leu Val Arg Lys Val Asp Gly
    210                 215                 220
Lys Ala Gly Leu Val Val His Ser Ser Met Glu Gln Asp Val Gly Leu
225                 230                 235                 240
Leu Arg Leu Tyr Pro Gly Ile Pro Ala Ala Leu Val Arg Ala Phe Leu
                245                 250                 255
Gln Pro Pro Leu Lys Gly Val Val Met Glu Thr Phe Gly Ser Gly Asn
            260                 265                 270
Gly Pro Thr Lys Pro Asp Leu Leu Gln Glu Leu Arg Val Ala Thr Glu
        275                 280                 285
Arg Gly Leu Val Ile Val Asn Cys Thr Gln Cys Leu Arg Gly Ala Val
    290                 295                 300
Thr Thr Asp Tyr Ala Ala Gly Met Ala Met Ala Gly Ala Gly Val Ile
305                 310                 315                 320
Ser Gly Phe Asp Met Thr Ser Glu Ala Ala Leu Ala Lys Leu Ser Tyr
                325                 330                 335
Val Leu Gly Gln Pro Gly Leu Ser Leu Asp Val Arg Lys Glu Leu Leu
            340                 345                 350
Thr Lys Asp Leu Arg Gly Glu Met Thr Pro Pro Ser Val
        355                 360                 365
```

SEQ ID NO: 8:

```
Met Ala Arg Ala Val Gly Pro Glu Arg Arg Leu Leu Ala Val Tyr Thr
1               5                  10                  15
Gly Gly Thr Ile Gly Met Arg Ser Glu Leu Gly Val Leu Val Pro Gly
            20                  25                  30
Thr Gly Leu Ala Ala Ile Leu Arg Thr Leu Pro Met Phe His Asp Glu
        35                  40                  45

Glu His Ala Arg Ala Arg Gly Leu Ser Glu Asp Thr Leu Val Leu Pro
    50                  55                  60
Pro Asp Ser Arg Asn Gln Arg Ile Leu Tyr Thr Val Leu Glu Cys Gln
65                  70                  75                  80
Pro Leu Phe Asp Ser Ser Asp Met Thr Ile Ala Glu Trp Val Arg Val
                85                  90                  95
Ala Gln Thr Ile Lys Arg His Tyr Glu Gln Tyr His Gly Phe Val Val
            100                 105                 110
Ile His Gly Thr Asp Thr Met Ala Phe Ala Ala Ser Met Leu Ser Phe
        115                 120                 125
Met Leu Glu Asn Leu Gln Lys Thr Val Ile Leu Thr Gly Ala Gln Val
    130                 135                 140
Pro Ile His Ala Leu Trp Ser Asp Gly Arg Glu Asn Leu Leu Gly Ala
145                 150                 155                 160
Leu Leu Met Ala Gly Gln Tyr Val Ile Pro Glu Val Cys Leu Phe Phe
                165                 170                 175
Gln Asn Gln Leu Phe Arg Gly Asn Arg Ala Thr Lys Val Asp Ala Arg
            180                 185                 190
Arg Phe Ala Ala Phe Cys Ser Pro Asn Leu Leu Pro Leu Ala Thr Val
        195                 200                 205
Gly Ala Asp Ile Thr Ile Asn Arg Glu Leu Val Arg Lys Val Asp Gly
    210                 215                 220
Lys Ala Gly Leu Val Val His Ser Ser Met Glu Gln Asp Val Gly Leu
225                 230                 235                 240
Leu Arg Leu Tyr Pro Gly Ile Pro Ala Ala Leu Val Arg Ala Phe Leu
                245                 250                 255
Gln Pro Pro Leu Lys Gly Val Val Met Glu Thr Phe Gly Ser Gly Asn
            260                 265                 270
Gly Pro Thr Lys Pro Asp Leu Leu Gln Glu Leu Arg Val Ala Thr Glu
        275                 280                 285
Arg Gly Leu Val Ile Val Asn Cys Thr Gln Cys Leu Gln Gly Ala Val
    290                 295                 300
Thr Thr Asp Tyr Ala Ala Gly Met Ala Met Ala Gly Ala Asn Val Ile
305                 310                 315                 320
Ser Gly Phe Asp Met Thr Ser Glu Ala Ala Leu Ala Lys Leu Ser Tyr
                325                 330                 335
Val Leu Gly Gln Pro Gly Leu Ser Leu Asp Val Arg Lys Glu Leu Leu
            340                 345                 350
Thr Lys Asp Leu Arg Gly Glu Met Thr Pro Pro Ser Val
        355                 360                 365
```

SEQ ID NO: 9:

```
Met Ala Arg Ala Val Gly Pro Glu Arg Arg Leu Leu Ala Val Tyr Thr
1               5                  10                  15
Gly Gly Thr Ile Gly Met Arg Ser Glu Leu Gly Val Leu Val Pro Gly
            20                  25                  30
Thr Gly Leu Ala Ala Ile Leu Arg Thr Leu Pro Met Phe His Asp Glu
        35                  40                  45
Glu His Ala Arg Ala Arg Gly Leu Ser Glu Asp Thr Leu Val Leu Pro
    50                  55                  60
Pro Asp Ser Arg Asn Gln Arg Ile Leu Tyr Thr Val Leu Glu Cys Gln
65                  70                  75                  80
Pro Leu Phe Asp Ser Ser Asp Met Thr Ile Ala Glu Trp Val Arg Val
                85                  90                  95

Ala Gln Thr Ile Lys Arg His Tyr Glu Gln Tyr His Gly Phe Val Val
            100                 105                 110
Ile His Gly Thr Asp Thr Met Ala Phe Ala Ala Ser Met Leu Ser Phe
        115                 120                 125
Met Leu Glu Asn Leu Gln Lys Thr Val Ile Leu Thr Gly Ala Gln Val
    130                 135                 140
Pro Ile His Ala Leu Trp Ser Asp Gly Arg Glu Asn Leu Leu Gly Ala
145                 150                 155                 160
Leu Leu Met Ala Gly Gln Tyr Val Ile Pro Glu Val Cys Leu Phe Phe
                165                 170                 175
Gln Asn Gln Leu Phe Arg Gly Asn Arg Ala Thr Lys Val Asp Ala Arg
            180                 185                 190
Arg Phe Ala Ala Phe Cys Ser Pro Asn Leu Leu Pro Leu Ala Thr Val
        195                 200                 205
Gly Ala Asp Ile Thr Ile Asn Arg Glu Leu Val Arg Lys Val Asp Gly
    210                 215                 220
Lys Ala Gly Leu Val Val His Ser Ser Met Glu Gln Asp Val Gly Leu
225                 230                 235                 240
Leu Arg Leu Tyr Pro Gly Ile Pro Ala Ala Leu Val Arg Ala Phe Leu
                245                 250                 255
Gln Pro Pro Leu Lys Gly Val Val Met Glu Thr Phe Gly Ser Gly Asn
            260                 265                 270
Gly Pro Thr Lys Pro Asp Leu Leu Gln Glu Leu Arg Val Ala Thr Glu
        275                 280                 285
Arg Gly Leu Val Ile Val Asn Cys Thr Gln Cys Leu Gln Gly Ala Val
    290                 295                 300
Thr Thr Asp Tyr Ala Ala Gly Met Ala Met Ala Gly Ala Gly Val Ile
305                 310                 315                 320
Ser Gly Phe Asp Met Thr Ser Glu Ala Ala Leu Ala Lys Leu Ser Tyr
                325                 330                 335
Val Leu Gly Gln Pro Gly Leu Ser Leu Asp Val Arg Lys Glu Leu Leu
            340                 345                 350
Thr Lys Asp Leu Arg Gly Glu Met Thr Pro Pro Ser Val.
        355                 360                 365
```

5. The polypeptide of claim 1, which originates from a member selected from the group consisting of guinea pig and human.

6. The polypeptide of claim 1, which exists in the form of an oligomer.

7. A DNA which encodes the polypeptide as claimed in claim 1.

8. The DNA of claim 7, which contains a nucleotide sequence selected from the group consisting of SEQ ID NOs:IO to 14, their homologous ones, and complementary ones thereunto;

SEQ ID NO:10

```
ATGGCGCGCG CATCAGGCTC CGAGAGGCAC CTGCTGCTCA TCTACACTGG CGGCACTTTG    60
GGCATGCAGA GCAAGGGCGG GGTGCTCGTC CCCGGCCCAG GCCTGGTCAC TCTGCTGCGG   120
ACCCTGCCCA TGTTCCATGA CAAGGAGTTC GCCCAGGCCC AGGGCCTCCC TGACCATGCT   180
CTGGCGCTGC CCCCTGCCAG CCACGGCCCC AGGGTCCTCT ACACGGTGCT GGAGTGCCAG   240
CCCCTCTTGG ATTCCAGCGA CATGACCATC GATGATTGGA TTCGCATAGC CAAGATCATA   300
GAGAGGCACT ATGAGCAGTA CCAAGGCTTT GTGGTTATCC ACGGCACCGA CACCATGGCC   360
TTTGGGGCCT CCATGCTGTC CTTCATGCTG GAAAACCTGC ACAAACCAGT CATCCTCACT   420
GGCGCCCAGG TGCCAATCCG TGTGCTGTGG AATGACGCCC GGGAAAACCT GCTGGGGGCG   480
TTGCTTGTGG CCGGCCAATA CATCATCCCT GAGGTCTGCC TGTTTATGAA CAGTCAGCTG   540
TTTCGGGGAA ACCGGGTAAC CAAGGTGGAC TCCCAGAAGT TTGAGGCCTT CTGCTCCCCC   600
AATCTGTCCC CACTAGCCAC TGTGGGCGCG GATGTCACAA TTGCCTGGGA CCTGGTGCGC   660
AAGGTCAACT GGAAGGACCC GCTGGTGGTG CACAGCAACA TGGAGCACGA CGTGGCACTG   720
CTGCGCCTCT ACCCTGGCAT CCCGGCCTCC CTGGTCCGGG CATTCCTGCA GCCCCCGCTC   780
AAGGGCGTGG TCCTGGAGAC CTTCGGCTCT GGCAACGGGC CGAGCAAGCC CGACCTGCTG   840
CAGGAGTTGC GGGCCGCGGC CCAGCGCGGC CTCATCATGG TCAACTGCAG CCAGTGCCTG   900
CGGGGGTCTG TGACCCCGGG CTATGCCACG AGCTTGGCGG GCGCCAACAT CGTGTCCGGC   960
TTAGACATGA CCTCAGAGGC CGCGCTGGCT AAGCTGTCCT ACGTGTTGGG CCTGCCGGAG  1020
CTGAGCCTGG AGCGCAGGCA GGAGCTGCTG GCCAAGGATC TTCGCGGGGA AATGACACTG  1080
CCCACGGCA                                                          1089
```

SEQ ID NO: 11:

```
ATGGCGCGCG CGGTGGGGCC CGAGCGGAGG CTGCTGGCCG TCTACACCGG CGGCACCATT    60
GGCATGCGGA GTGAGCTCGG CGTGCTTGTG CCCGGGACGG GCCTGGCTGC CATCCTGAGG   120
ACACTGCCCA TGTTCCATGA CGAGGAGCAC GCCCGAGCCC GCGGCCTCTC TGAGGACACC   180
CTGGTGCTAC CCCCGGACAG CCGCAACCAG AGGATCCTCT ACACCGTGCT GGAGTGCCAG   240
CCCCTCTTCG ACTCCAGTGA CATGACCATC GCTGAGTGGG TTCGCGTTGC CCAGACCATC   300
AAGAGGCACT ACGAGCAGTA CCACGGCTTT GTGGTCATCC ACGGCACCGA CACCATGGCC   360
TTTGCTGCCT CGATGCTGTC CTTCATGCTG GAGAACCTGC AGAAGACTGT CATCCTCACT   420
GGGGCCCAGG TGCCCATCCA TGCCCTGTGG AGCGACGGCC GTGAGAACCT GCTGGGGGCA   480
CTGCTCATGG CTGGCCAGTA TGTGATCCCA GAGGTCTGCC TTTTCTTCCA GAATCAGCTG   540
TTTCGGGGCA ACCGGGCAAC CAAGGTAGAC GCTCGGAGGT TCGCAGCTTT CTGCTCCCCG   600
AACCTGCTGC CTCTGGCCAC AGTGGGTGCT GACATCACAA TCAACAGGGA GCTGGTGCGG   660
AAGGTGGACG GGAAGGCTGG GCTGGTGGTG CACAGCAGCA TGGAGCAGGA CGTGGGCCTG   720
CTGCGCCTCT ACCCTGGGAT CCCTGCCGCC CTGGTTCGGG CCTTCTTGCA GCCTCCCCTG   780
AAGGGCGTGG TCATGGAGAC CTTCGGTTCA GGGAACGGAC CCACCAAGCC CGACCTGCTG   840
CAGGAGCTGC GGGTGGCCAC CGAGCGCGGC CTGGTCATCG TCAACTGTAC CCAGTGCCTC   900
CGGGGGGCTG TGACCACAGA CTATGCAGCT GGCATGGCCA TGGCGGGAGC CAACGTCATC   960
TCAGGCTTCG ACATGACATC GGAGGCCGCC CTGGCCAAGC TATCGTATGT GCTGGGCCAG  1020
CCAGGGCTGA GCCTGGATGT CAGGAAGGAG CTGCTGACCA AGGACCTTCG GGGGGAGATG  1080
ACGCCACCCT CGGTG                                                   1095
```

SEQ ID NO: 12:

```
ATGGCGCGCG CGGTGGGGCC CGAGCGGAGG CTGCTGGCCG TCTACACCGG CGGCACCATT   60
GGCATGCGGA GTGAGCTCGG CGTGCTTGTG CCCGGGACGG GCCTGGCTGC CATCCTGAGG  120
ACACTGCCCA TGTTCCATGA CGAGGAGCAC GCCCGAGCCC GCGGCCTCTC TGAGGACACC  180
CTGGTGCTAC CCCCGGACAG CCGCAACCAG AGGATCCTCT ACACCGTGCT GGAGTGCCAG  240
CCCCTCTTCG ACTCCAGTGA CATGACCATC GCTGAGTGGG TTCGCGTTGC CCAGACCATC  300
AAGAGGCACT ACGAGCAGTA CCACGGCTTT GTGGTCATCC ACGGCACCGA CACCATGGCC  360

TTTGCTGCCT CGATGCTGTC CTTCATGCTG GAGAACCTGC AGAAGACTGT CATCCTCACT  420
GGGGCCCAGG TGCCCATCCA TGCCCTGTGG AGCGACGGCC GTGAGAACCT GCTGGGGGCA  480
CTGCTCATGG CTGGCCAGTA TGTGATCCCA GAGGTCTGCC TTTTCTTCCA GAATCAGCTG  540
TTTCGGGGCA ACCGGGCAAC CAAGGTAGAC GCTCGGAGGT TCGCAGCTTT CTGCTCCCCG  600
AACCTGCTGC CTCTGGCCAC AGTGGGTGCT GACATCACAA TCAACAGGGA GCTGGTGCGG  660
AAGGTGGACG GGAAGGCTGG GCTGGTGGTG CACAGCAGCA TGGAGCAGGA CGTGGGCCTG  720
CTGCGCCTCT ACCCTGGGAT CCCTGCCGCC CTGGTTCGGG CCTTCTTGCA GCCTCCCCTG  780
AAGGGCGTGG TCATGGAGAC CTTCGGTTCA GGGAACGGAC CCACCAAGCC CGACCTGCTG  840
CAGGAGCTGC GGGTGGCCAC CGAGCGCGGC CTGGTCATCG TCAACTGTAC CCAGTGCCTC  900
CGGGGGGCTG TGACCACAGA CTATGCAGCT GGCATGGCCA TGGCGGGAGC CGGCGTCATC  960
TCAGGCTTCG ACATGACATC GGAGGCCGCC CTGGCCAAGC TATCGTATGT GCTGGGCCAG 1020
CCAGGGCTGA GCCTGGATGT CAGGAAGGAG CTGCTGACCA AGGACCTTCG GGGGGAGATG 1080
ACGCCACCCT CGGTG                                                   1095
```

SEQ ID NO: 13:

```
ATGGCGCGCG CGGTGGGGCC CGAGCGGAGG CTGCTGGCCG TCTACACCGG CGGCACCATT   60
GGCATGCGGA GTGAGCTCGG CGTGCTTGTG CCCGGGACGG GCCTGGCTGC CATCCTGAGG  120
ACACTGCCCA TGTTCCATGA CGAGGAGCAC GCCCGAGCCC GCGGCCTCTC TGAGGACACC  180
CTGGTGCTAC CCCCGGACAG CCGCAACCAG AGGATCCTCT ACACCGTGCT GGAGTGCCAG  240
CCCCTCTTCG ACTCCAGTGA CATGACCATC GCTGAGTGGG TTCGCGTTGC CCAGACCATC  300
AAGAGGCACT ACGAGCAGTA CCACGGCTTT GTGGTCATCC ACGGCACCGA CACCATGGCC  360
TTTGCTGCCT CGATGCTGTC CTTCATGCTG GAGAACCTGC AGAAGACTGT CATCCTCACT  420
GGGGCCCAGG TGCCCATCCA TGCCCTGTGG AGCGACGGCC GTGAGAACCT GCTGGGGGCA  480
CTGCTCATGG CTGGCCAGTA TGTGATCCCA GAGGTCTGCC TTTTCTTCCA GAATCAGCTG  540
TTTCGGGGCA ACCGGGCAAC CAAGGTAGAC GCTCGGAGGT TCGCAGCTTT CTGCTCCCCG  600
AACCTGCTGC CTCTGGCCAC AGTGGGTGCT GACATCACAA TCAACAGGGA GCTGGTGCGG  660
AAGGTGGACG GGAAGGCTGG GCTGGTGGTG CACAGCAGCA TGGAGCAGGA CGTGGGCCTG  720
CTGCGCCTCT ACCCTGGGAT CCCTGCCGCC CTGGTTCGGG CCTTCTTGCA GCCTCCCCTG  780
AAGGGCGTGG TCATGGAGAC CTTCGGTTCA GGGAACGGAC CCACCAAGCC CGACCTGCTG  840
CAGGAGCTGC GGGTGGCCAC CGAGCGCGGC CTGGTCATCG TCAACTGTAC CCAGTGCCTC  900
CAGGGGGCTG TGACCACAGA CTATGCAGCT GGCATGGCCA TGGCGGGAGC CAACGTCATC  960
TCAGGCTTCG ACATGACATC GGAGGCCGCC CTGGCCAAGC TATCGTATGT GCTGGGCCAG 1020
CCAGGGCTGA GCCTGGATGT CAGGAAGGAG CTGCTGACCA AGGACCTTCG GGGGGAGATG 1080
ACGCCACCCT CGGTG                                                   1095
```

SEQ ID NO: 14:

```
ATGGCGCGCG CGGTGGGGCC CGAGCGGAGG CTGCTGGCCG TCTACACCGG CGGCACCATT   60
GGCATGCGGA GTGAGCTCGG CGTGCTTGTG CCCGGGACGG GCCTGGCTGC CATCCTGAGG  120
ACACTGCCCA TGTTCCATGA CGAGGAGCAC GCCCGAGCCC GCGGCCTCTC TGAGGACACC  180
CTGGTGCTAC CCCCGGACAG CCGCAACCAG AGGATCCTCT ACACCGTGCT GGAGTGCCAG  240
CCCCTCTTCG ACTCCAGTGA CATGACCATC GCTGAGTGGG TTCGCGTTGC CCAGACCATC  300
AAGAGGCACT ACGAGCAGTA CCACGGCTTT GTGGTCATCC ACGGCACCGA CACCATGGCC  360
TTTGCTGCCT CGATGCTGTC CTTCATGCTG GAGAACCTGC AGAAGACTGT CATCCTCACT  420
GGGGCCCAGG TGCCCATCCA TGCCCTGTGG AGCGACGGCC GTGAGAACCT GCTGGGGGCA  480
CTGCTCATGG CTGGCCAGTA TGTGATCCCA GAGGTCTGCC TTTTCTTCCA GAATCAGCTG  540
TTTCGGGGCA ACCGGGCAAC CAAGGTAGAC GCTCGGAGGT TCGCAGCTTT CTGCTCCCCG  600
AACCTGCTGC CTCTGGCCAC AGTGGGTGCT GACATCACAA TCAACAGGGA GCTGGTGCGG  660
AAGGTGGACG GGAAGGCTGG GCTGGTGGTG CACAGCAGCA TGGAGCAGGA CGTGGGCCTG  720
CTGCGCCTCT ACCCTGGGAT CCCTGCCGCC CTGGTTCGGG CCTTCTTGCA GCCTCCCCTG  780
AAGGGCGTGG TCATGGAGAC CTTCGGTTCA GGGAACGGAC CCACCAAGCC CGACCTGCTG  840
CAGGAGCTGC GGGTGGCCAC CGAGCGCGGC CTGGTCATCG TCAACTGTAC CCAGTGCCTC  900

CAGGGGGCTG TGACCACAGA CTATGCAGCT GGCATGGCCA TGGCGGGAGC CGGCGTCATC  960
TCAGGCTTCG ACATGACATC GGAGGCCGCC CTGGCCAAGC TATCGTATGT GCTGGGCCAG 1020
CCAGGGCTGA GCCTGGATGT CAGGAAGGAG CTGCTGACCA AGGACCTTCG GGGGGAGATG 1080
ACGCCACCCT CGGTG                                                   1095.
```

9. The DNA of claim 7, which originates from a member selected from the group consisting of guinea pig and human.

10. A self-replicable vector which contains a DNA encoding the polypeptide as claimed in claim 1.

11. The self-replicable vector of claim 10, which is a plasmid vector.

12. The self-replicable vector of claim 10, which contains one or more members selected from the group consisting of metallothionein and Tac promotors.

13. A transformant obtainable by introducing the DNA of claim 7 into a host.

14. The transformant of claim 13, wherein said host is a nenber selected from the group consisting of prokaryotic and eukaryotic cells.

15. The transformant of claim 13, wherein said host is *Escherichia coli.*

16. The transformant of claim 13, wherein said host is a mouse cell.

17. A transformant obtainable by the self replicable vector of claim 10 into a host.

18. The transformant of claim 17, wherein said host is a member selected from the group consisting of prokaryotic and eukaryotic cells.

19. The transformant of claim 17, wherein said host is *Escherichia coli.*

20. The transformant of claim 17, wherein said host is a mouse cell.

21. A process for preparing a polypeptide, which comprises (a) artificially expressing a DNA encoding the polypeptide of claim 1, and (b) collecting the polypeptide from the resultant mixture.

22. The process of claim 21, wherein the artificial expression of the step (a) contains culturing the transformant of

claim 13.

23. The process of claim 21, wherein the artificial expression of the step (a) contains culturing the transformant of claim 17.

24. The process of claim 21, wherein the resultant mixture of the step (b) is a culture of the transformant of claim 13.

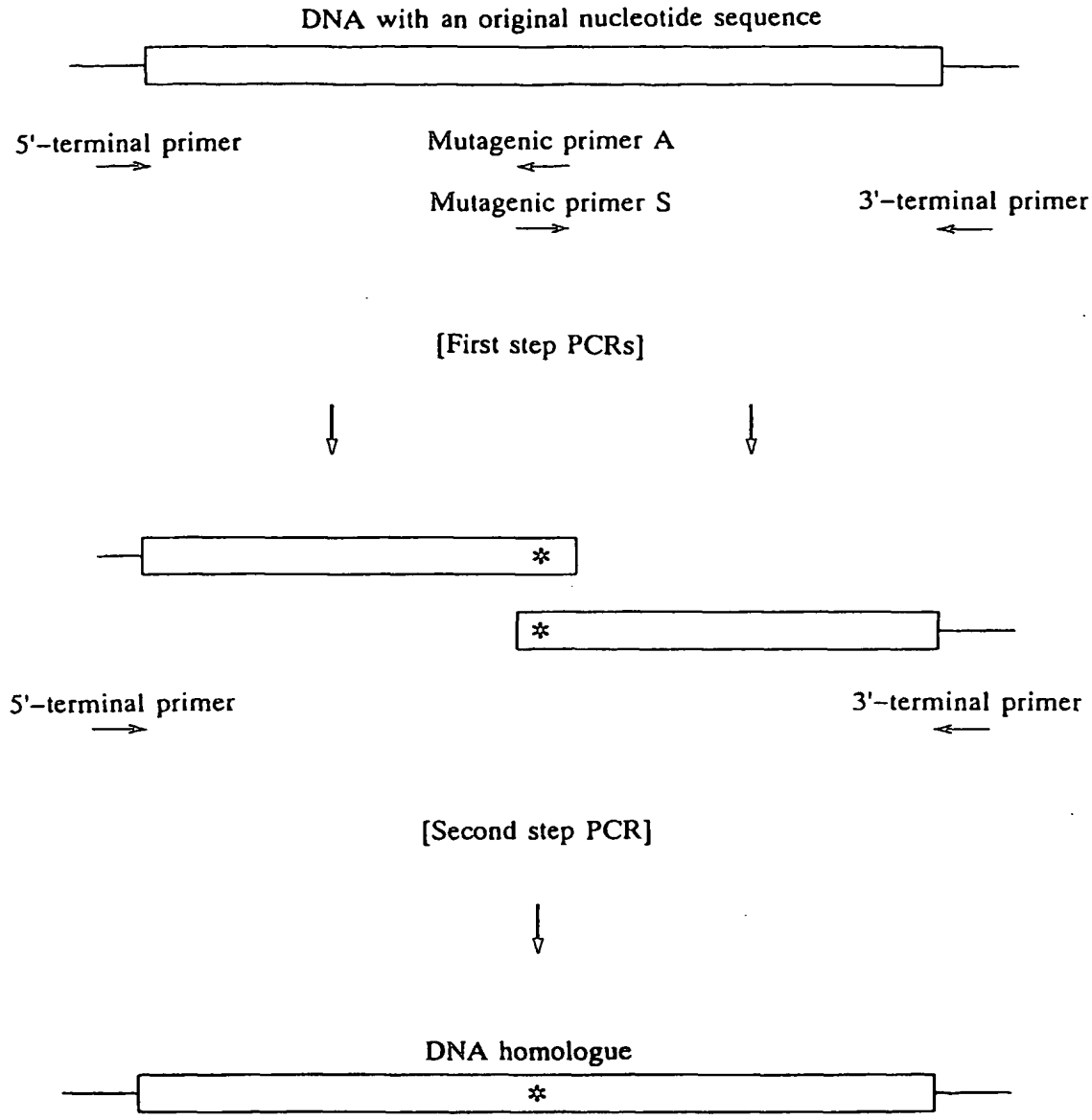
25. The process of claim 21, wherein the resultant mixture of the step (b) is a culture of the transformant of claim 17.

26. The process of claim 21, wherein the polypeptide is collected by one or more techniques selected from the group consisting of salting out, dialysis, filtration, concentration, gel filtration chromatography, ion-exchange chromatography, affinity chromatography, hydrophobic chromatography, isoelectric focusing and gel electrophoresis.

27. An agent for susceptive diseases, which contains the polypeptide of claim 1 as an effective ingredient.

28. The agent of claim 27, wherein said diseases are malignant tumors, leukemias and lymphomas.

29. The agent of claim 27, which contains one or more members selected from the group consisting of serum albumin, glycerol, gelatin, trehalose and maltose as a stabilizer.



Note: An asterisk indicates a site where a nucleotide is substituted, and a box indicates a polypeptide-encoding sequence.

FIG.1

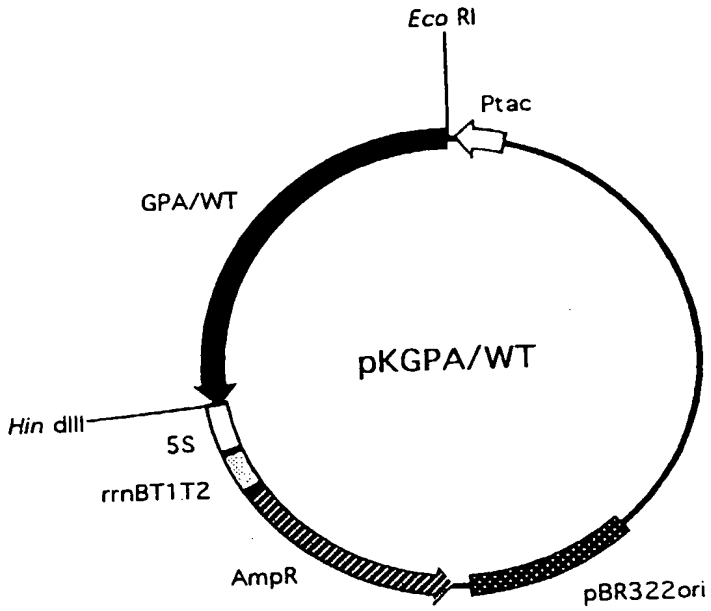


FIG.2

Template DNA : pCGPA/WT
Sense primer : 5'-GTGAATTCGGAGGTTCAGATGGCGCGCGCATCA-3'
Anti-sense primer : 5'-CTGCGGCCGCTCAGATGGCAGGCGGCAC-3'

↓ PCR

Amplified DNA

↓ Cleavage by *Eco* RI and *Not* I

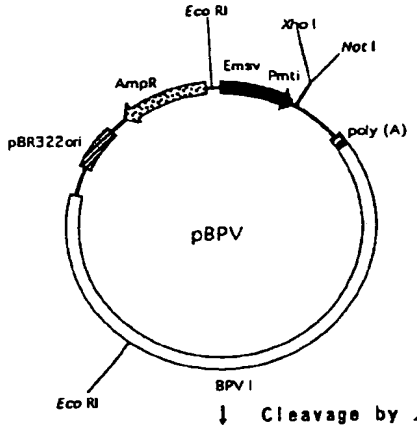
DNA fragment about 1.7 kbp in length

Linkers :

5'-TCGAGCCACCATGAAGTGTTCGTGGGTTATT-3'
5'-TTCTTCCTGATGGCCGTAGTGACAGGAGTG-3'
5'-AATTCACTCCTGTCACTACGGCCATCAGGA-3'
5'-AGAAAATAACCCACGAACACTTCATGGTGGC-3'

↓ Phoshporylation by T4 polynucleotide kinase

5'-terminal phosphorylated linkers



↓ Cleavage by *Xho* I and *Not* I

Ligation

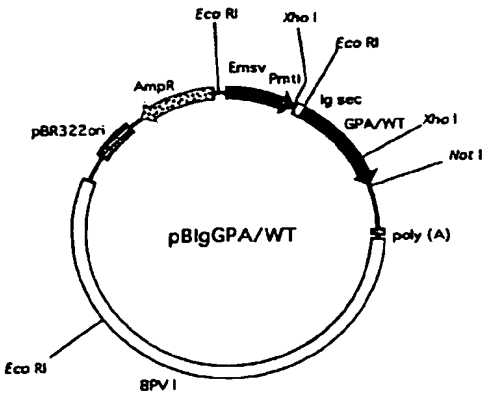


FIG.3

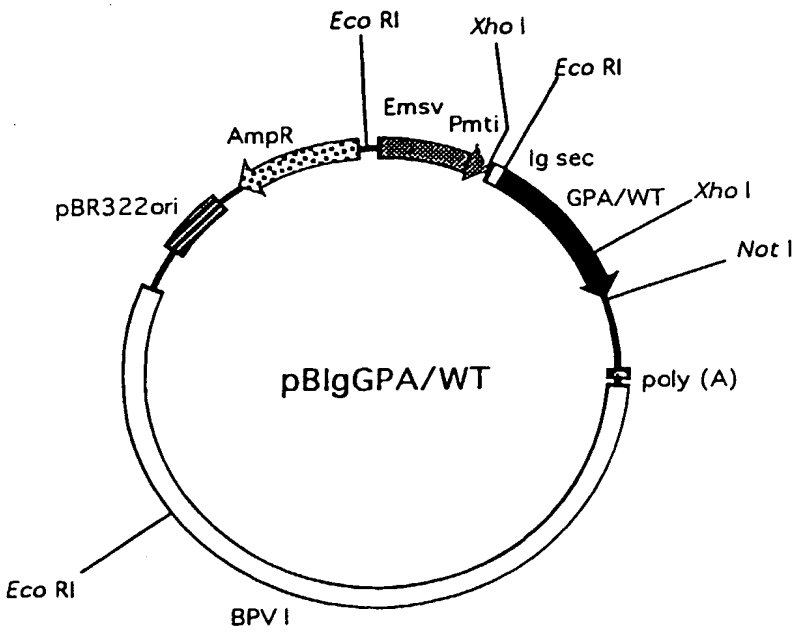


FIG.4

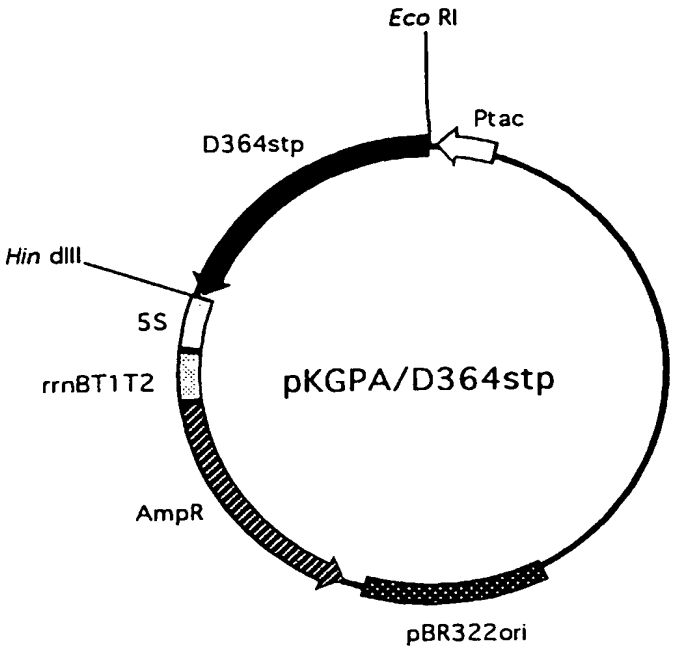


FIG.5

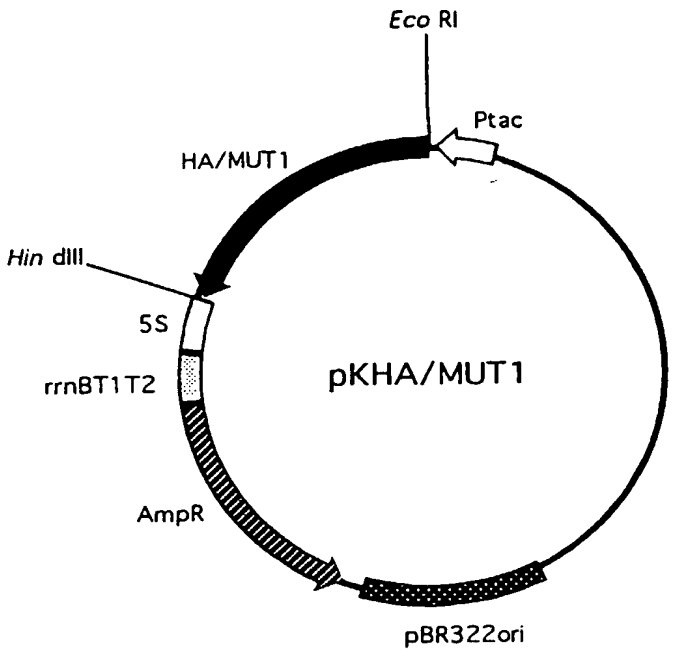


FIG.6

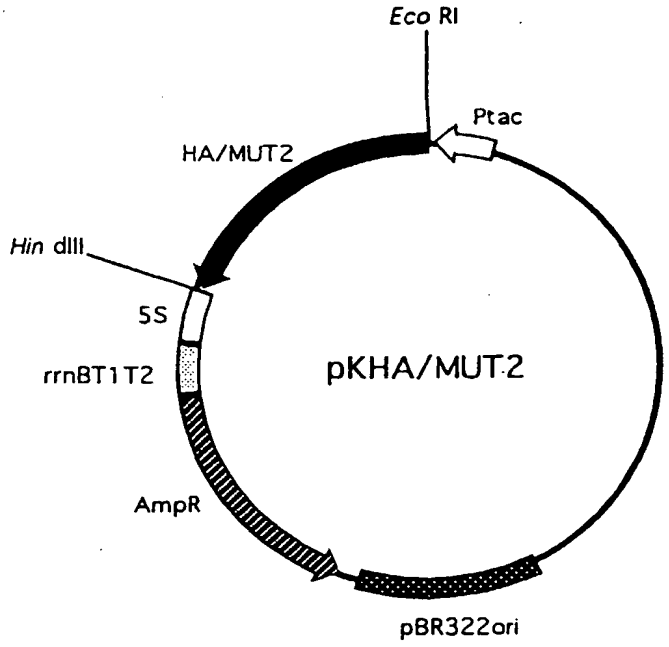


FIG.7

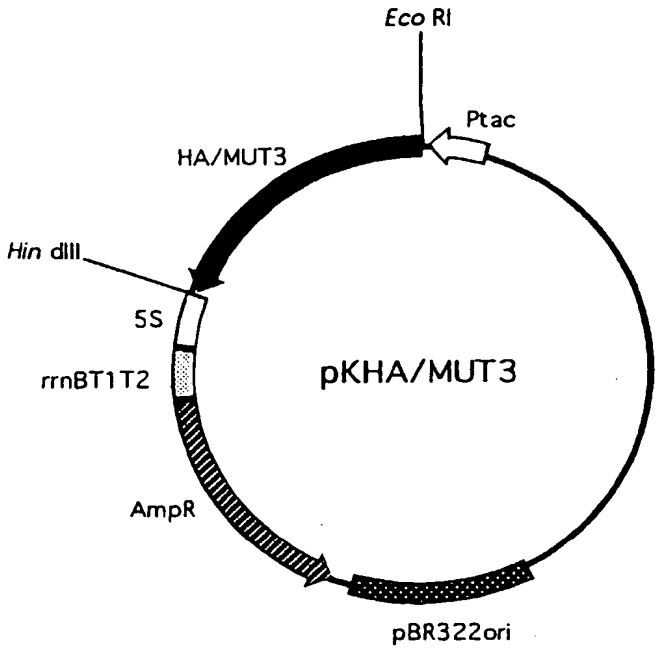


FIG.8

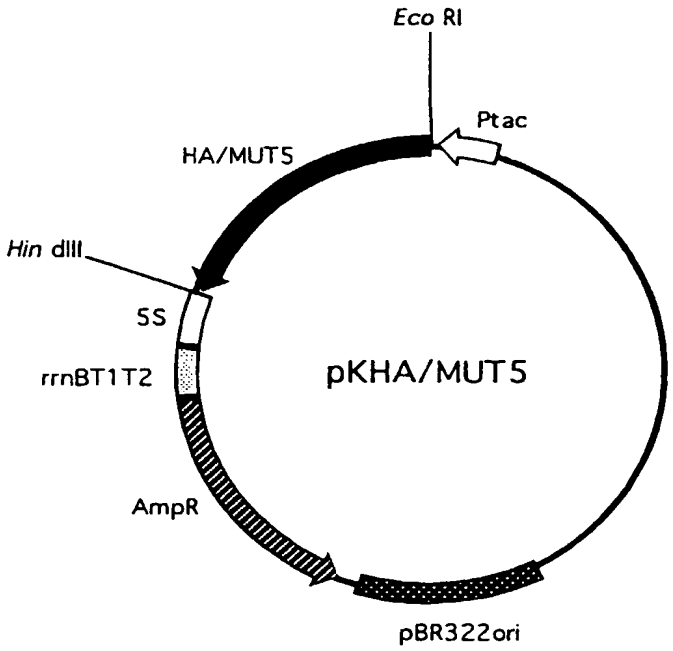


FIG.9

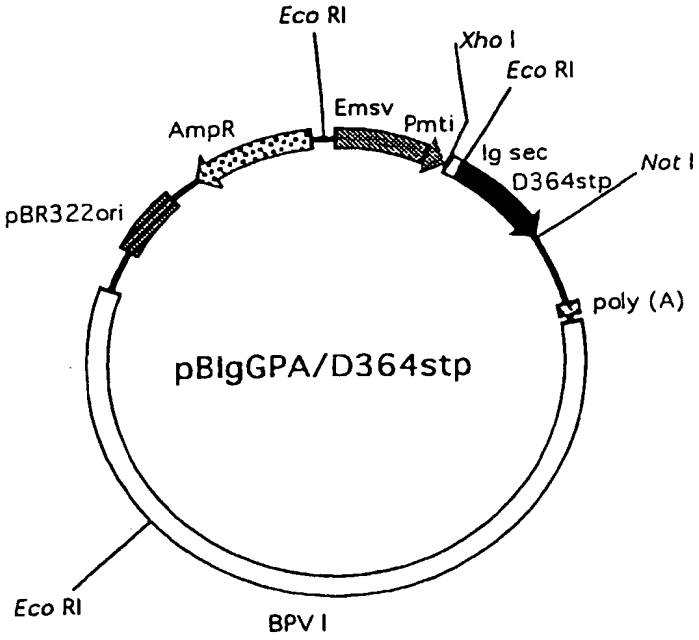


FIG.10

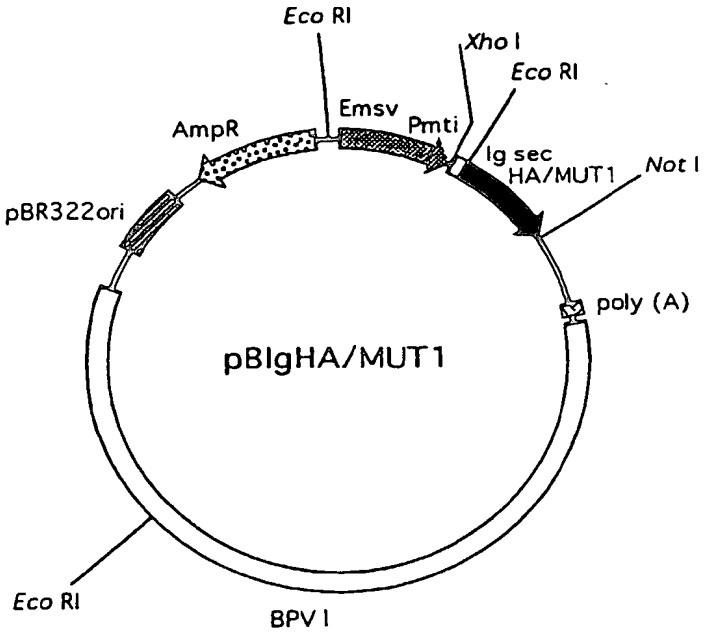


FIG.11

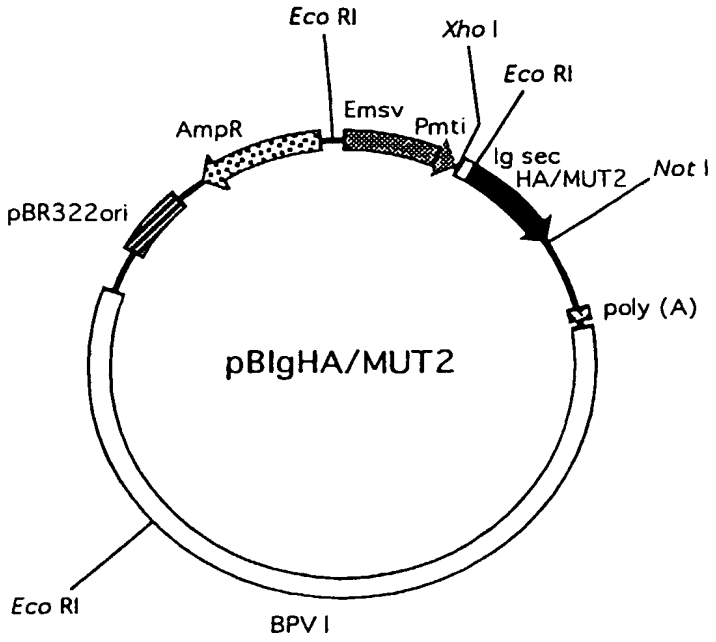


FIG.12

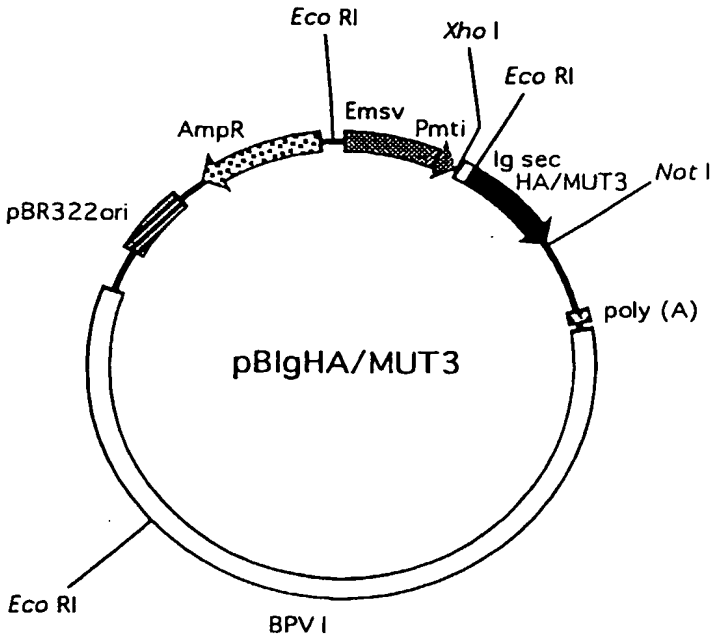


FIG.13

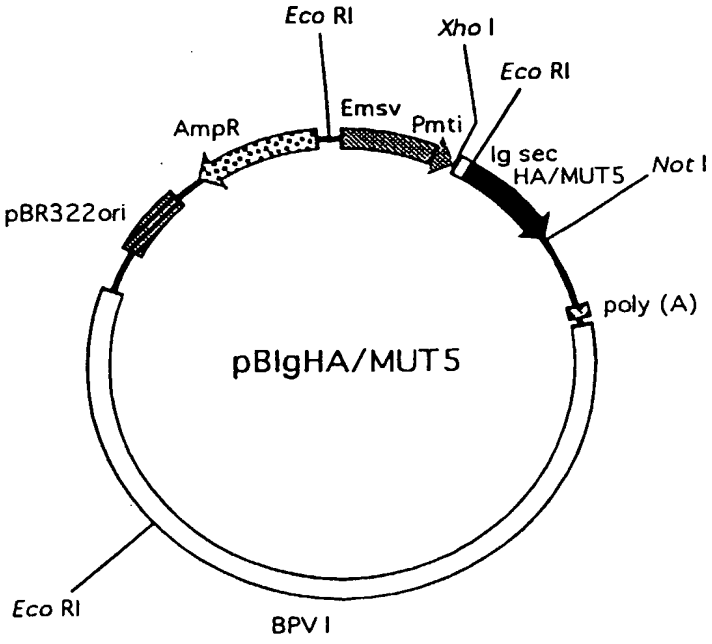


FIG.14

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 811 687 A3

(12) EUROPEAN PATENT APPLICATION

(88) Date of publication A3:
28.10.1998 Bulletin 1998/44

(43) Date of publication A2:
10.12.1997 Bulletin 1997/50

(21) Application number: **97303896.1**

(22) Date of filing: **06.06.1997**

(51) Int Cl.[6]: **C12N 15/55**, C12N 9/82, C12N 15/70, C12N 15/79, C12N 1/21, C12N 5/10, A61K 38/46
// (C12N1/21, C12R1:19)

(84) Designated Contracting States:
AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV RO SI

(30) Priority: **07.06.1996 JP 168172/96**

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(54) Polypeptides having l-asparaginase activity

(57) Disclosed are polypeptides which originate from mammal, having L-asparaginase activity. The polypeptides are easily prepared by applying recombinant DNA techniques to DNAs encoding the polypeptides and they exert satisfactory effects in the treatment and/or the prevention for diseases caused by tumor cells dependent on L-asparagine, and cause no substantial serious side effects even when administered to humans in relatively-high dose.

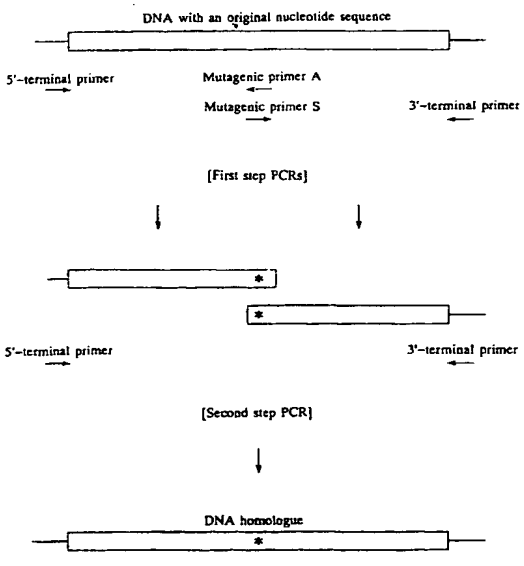

FIG.1

EP 0 811 687 A3

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 97 30 3896

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X,P	EP 0 726 313 A (HAYASHIBARA BIOCHEM LAB) 14 August 1996 * the whole document *	1-11, 13-15, 17-19, 21-25, 27,28	C12N15/55 C12N9/82 C12N15/70 C12N15/79 C12N1/21 C12N5/10 A61K38/46
X	YELLIN T O ET AL: "PURIFICATION AND PROPERTIES OF GUINIEA PIG SERUM ASPARAGINASE" BIOCHEMISTRY, vol. 5, no. 5, May 1966, pages 1605-1612, XP002012974 * the whole document *	1-29	//(C12N1/21, C12R1:19)
X	PATENT ABSTRACTS OF JAPAN vol. 004, no. 046 (C-006), 10 April 1980 & JP 55 019018 A (TOUBISHI YAKUHIN KOGYO KK), 9 February 1980 * abstract *	1,2,4-29	
X	PATENT ABSTRACTS OF JAPAN vol. 017, no. 158 (C-1041), 29 March 1993 & JP 04 320684 A (JAPAN SYNTHETIC RUBBER CO LTD), 11 November 1992 * abstract *	1,2,4-29	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)
C12N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	7 September 1998	Fernandez y Branas,F

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D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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